MSQ Plus™

Chromeleon® / MSQ Plus Operator's Guide

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DIONEX

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Chromeleon / MSQ Plus Operator's Guide

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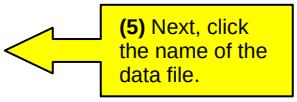
Chromeleon / MSQ Plus Operator's Manual

1.1 Introduction

The intent of this manual is to familiarize users with basic concepts of MSQ operation under Chromeleon control. Users should already be comfortable with the hardware of the front-end ion chromatograph (IC) or liquid chromatograph (LC), MSQ Plus, and with basic Chromeleon functions. This manual should not be used as a substitute for formal Chromeleon training.

1.2 Conventions

The figures and call-outs used in this manual are to aid the user in performing

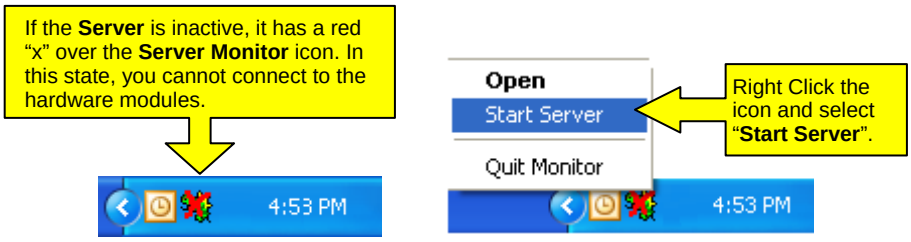
Directions and actions	
Directions to be performed in an ordered sequence	
Alternate ways of performing a given task	
Commonly used commands/icons for Mass Spectrometry	
Important Notes and Cautions	**Caution: Always turn power OFF**

The Chromeleon Server Monitor

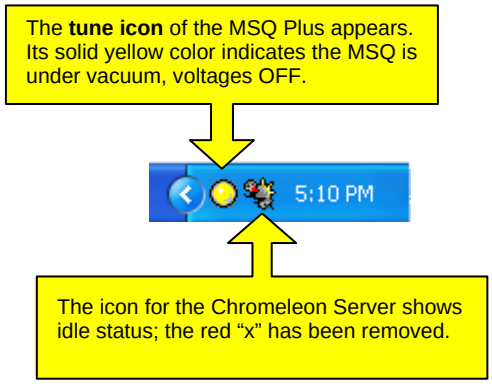
2.1 Starting the Server

When the computer is initially started, the Chromeleon Server is “not running”. In order to establish communication with the devices, including the mass spectrometer, open the Server Monitor.

1. Locate the Server Monitor in the task bar.
2. Identify the status of the Server.
    a. If the server is “not running”, the server monitor appears as a small green “Chromeleon” caricature with a red cross over the top.
    b. Right click on the server monitor icon and “start server”.


If the **Server** is inactive, it has a red “x” over the **Server Monitor** icon. In this state, you cannot connect to the hardware modules.
4:53 PM
Open
Start Server
Quit Monitor
Right Click the icon and select “**Start Server**”.
4:53 PM

    c. When the server is running idle, the red cross disappears from the Chromeleon icon and the tune icon for the MSQ Plus™ mass spectrometer appears in the task bar.


The **tune icon** of the MSQ Plus appears. Its solid yellow color indicates the MSQ is under vacuum, voltages OFF.
5:10 PM
The icon for the Chromeleon Server shows idle status; the red “x” has been removed.

3. The Server must be on and idle before proceeding. The tune monitor for the MSQ Plus shows the status of the mass spectrometer. It automatically appears when the Chromeleon Server is started. The color of the icon displays the status – it is red when the instrument is vented, flashing yellow during pump down. The icon must show a solid yellow (pumped/idle) or green (active) circle before you can access the mass spectrometer.

Chromeleon Software

3.1 Starting Chromeleon

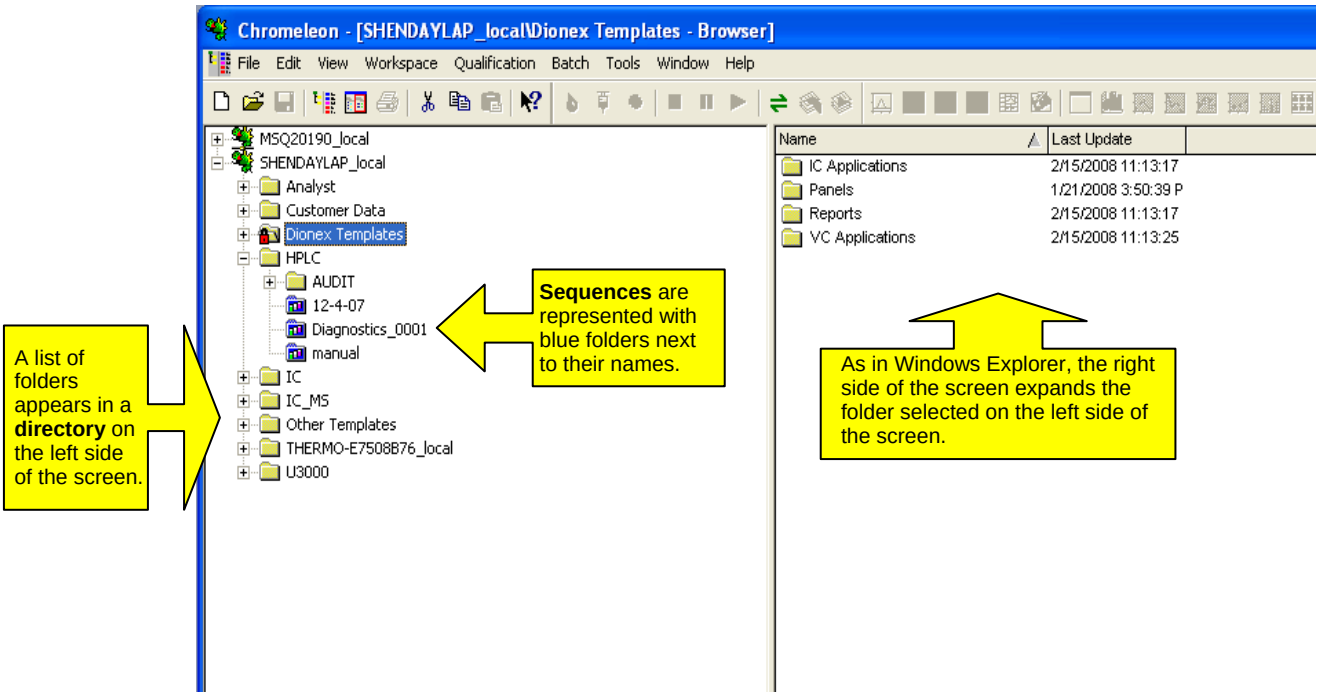
Enter all commands for the chromatography system through Chromeleon software.

1. Locate the Chromeleon icon on the Desktop of the computer.

2. Double click the icon.
3. The software is configured to reveal either the Browser or the Panel Tabset.

3.2 The Browser

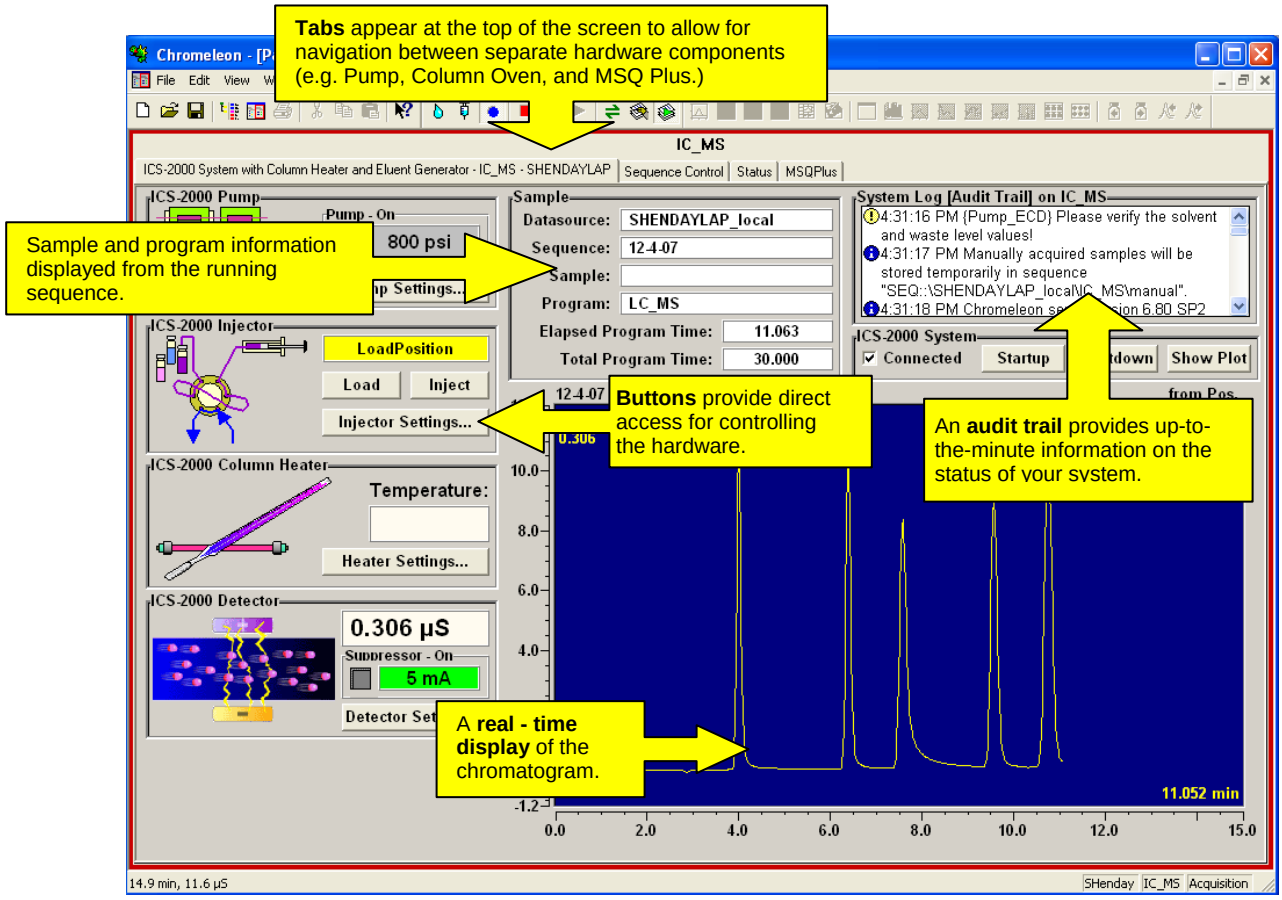
The Browser is designed to mirror Windows Explorer. Use the Browser to navigate to data files, hardware programs, and quantification methods.



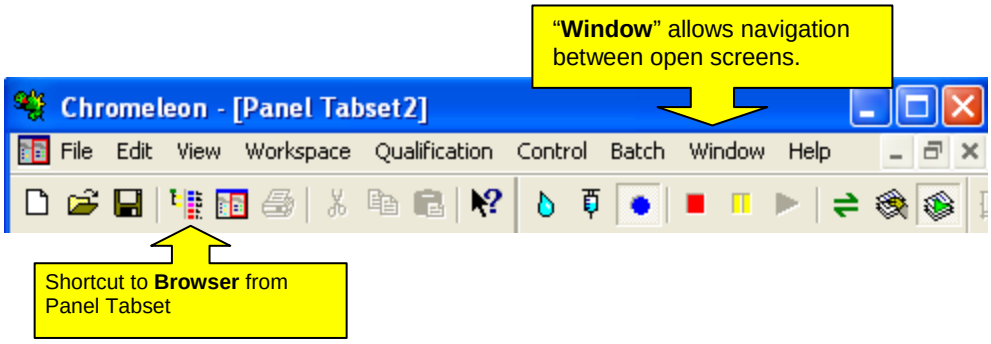
3.3 The Panel Tabset

The Panel Tabset is designed to give the user access to direct control over the chromatography system. Each panel tabset is unique for the hardware configuration.

> The Panel Tabsets shown in the following figures may not represent the actual screens on your computer; however, all statements are fully transferable.



Familiarize yourself with the menus and toolbars at the top of the screen to navigate between these windows.

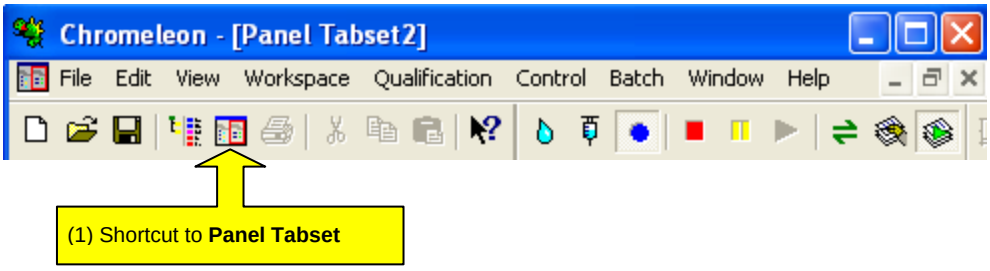

"**Window**" allows navigation between open screens.
Chromeleon - [Panel Tabset2]
File Edit View Workspace Qualification Control Batch Window Help
Shortcut to **Browser** from Panel Tabset

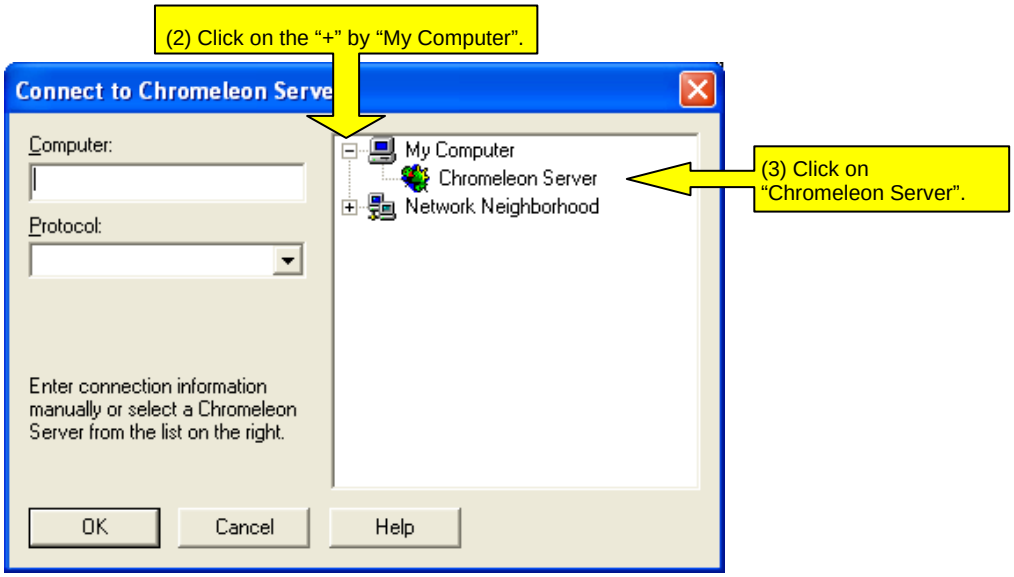
3.4 Creating/Modifying the Panel Tabset

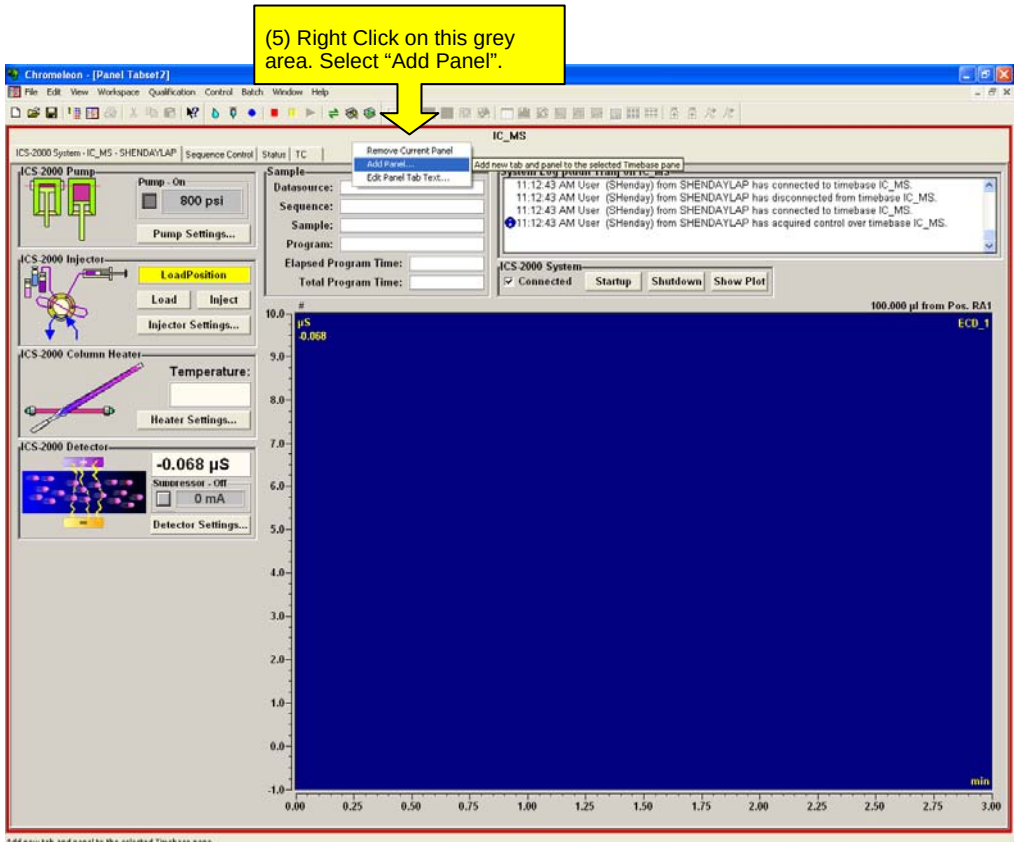
Your Dionex Service Representative should have created a custom Panel Tabset for your system. A link to this tabset should be found in your Browser.

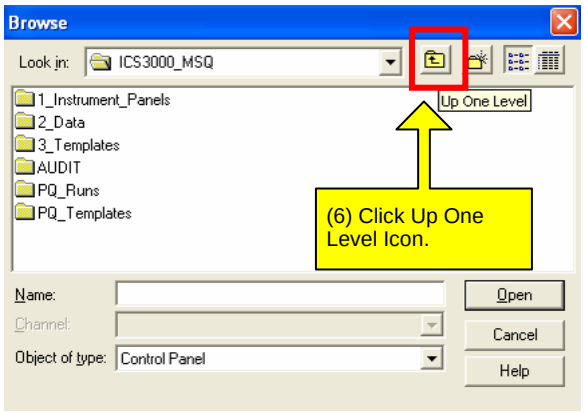
To create your own Panel Tabset:

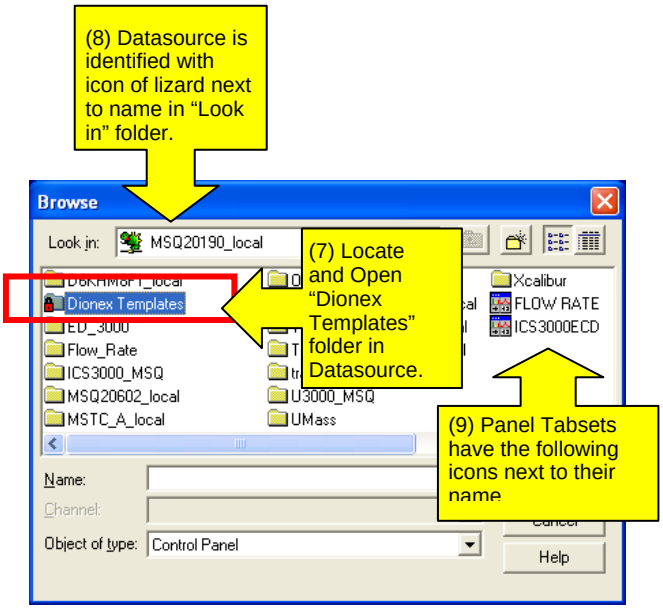
1. Click on the shortcut to the Panel Tabset icon or as in the yellow box, Click on the Shortcut to Panel Tabset icon.
2. To connect to the Chromeleon Server, click on the "+" by "My Computer".
3. Click on the Chromeleon Server to populate the "Computer" and "Protocol" field on the left side of the box.
4. Notice the Panel Tabset appears without the tab for the MSQ.
5. Right click on the grey space to the right of the last tab and select "Add Panel…".
6. Click the "Up One Level" icon on the pop-up screen until you get to the Datasource name.
7. Open the "Dionex Templates" folder. This folder typically has a red lock next to the name.
8. Browse to and open the "Panels" folder inside "Dionex Templates".
9. Once inside the "Panels" folder, open the "LC_MS" folder.
10. Double click on the "MS_SIM_Mode" panel.
11. The panel is added to your tabset.
12. Save the tabset in a location you will later be able to find.
13. The next time this tabset is open, it will include the MSQ tab.

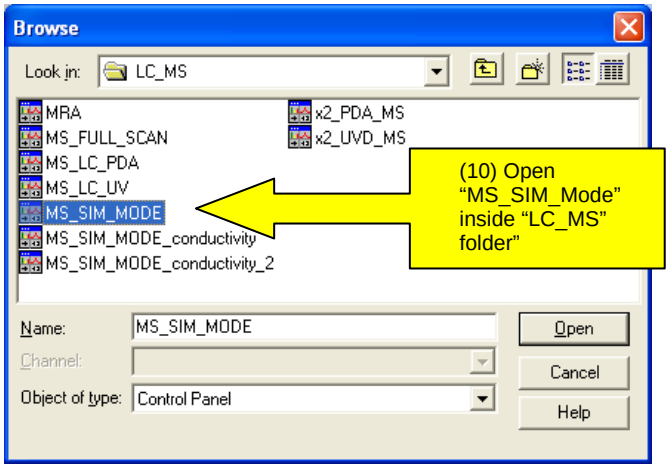

Chromeleon - [Panel Tabset2]
File Edit View Workspace Qualification Control Batch Window Help
(1) Shortcut to **Panel Tabset**

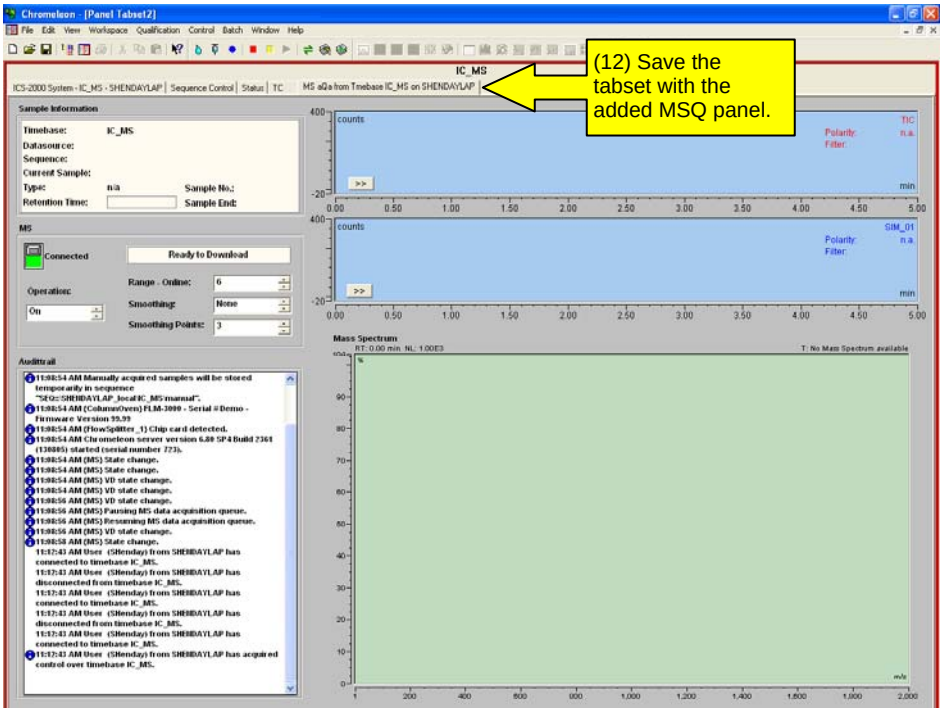

(2) Click on the "+" by "My Computer".
Connect to Chromeleon Serve
Computer:
Protocol:
Enter connection information manually or select a Chromeleon Server from the list on the right.
My Computer
Chromeleon Server
Network Neighborhood
(3) Click on "Chromeleon Server".
OK
Cancel
Help


(5) Right Click on this grey area. Select "Add Panel".
Chromeleon - [Panel Tabset2]
IC_MS
Remove Current Panel
Add Panel...
Edit Panel Tab Text...
ICS-2000 Pump
Pump On
800 psi
Pump Settings...
ICS-2000 Injector
LoadPosition
Load
Inject
Injector Settings...
ICS-2000 Column Heater
Temperature:
Heater Settings...
ICS-2000 Detector
-0.068 µS
Suppressor - Off
0 mA
Detector Settings...
Sample
Datasource:
Sequence:
Sample:
Program:
Elapsed Program Time:
Total Program Time:
ICS-2000 System
Connected
Startup
Shutdown
Show Plot
100.000 µl from Pos. RA1
ECD_1
min


Browse
Look in: ICS3000_MSQ
Up One Level
1_Instrument_Panels
2_Data
3_Templates
AUDIT
PQ_Runs
PQ_Templates
(6) Click Up One Level Icon.
Name:
Channel:
Object of type: Control Panel
Open
Cancel
Help


(8) Datasource is identified with icon of lizard next to name in "Look in" folder.
Browse
Look in: MSQ20190_local
Dionex Templates
(7) Locate and Open "Dionex Templates" folder in Datasource.
Flow_Rate
ICS3000_MSQ
MSQ20602_local
MSTC_A_local
U3000_MSQ
UMass
Xcalibur
FLOW RATE
ICS3000ECD
(9) Panel Tabsets have the following icons next to their name
Name:
Channel:
Object of type: Control Panel
Help


Browse
Look in: LC_MS
MRA
MS_FULL_SCAN
MS_LC_PDA
MS_LC_UV
MS_SIM_MODE
MS_SIM_MODE_conductivity
MS_SIM_MODE_conductivity_2
x2_PDA_MS
x2_UVD_MS
(10) Open "MS_SIM_Mode" inside "LC_MS" folder"
Name: MS_SIM_MODE
Channel:
Object of type: Control Panel
Open
Cancel
Help

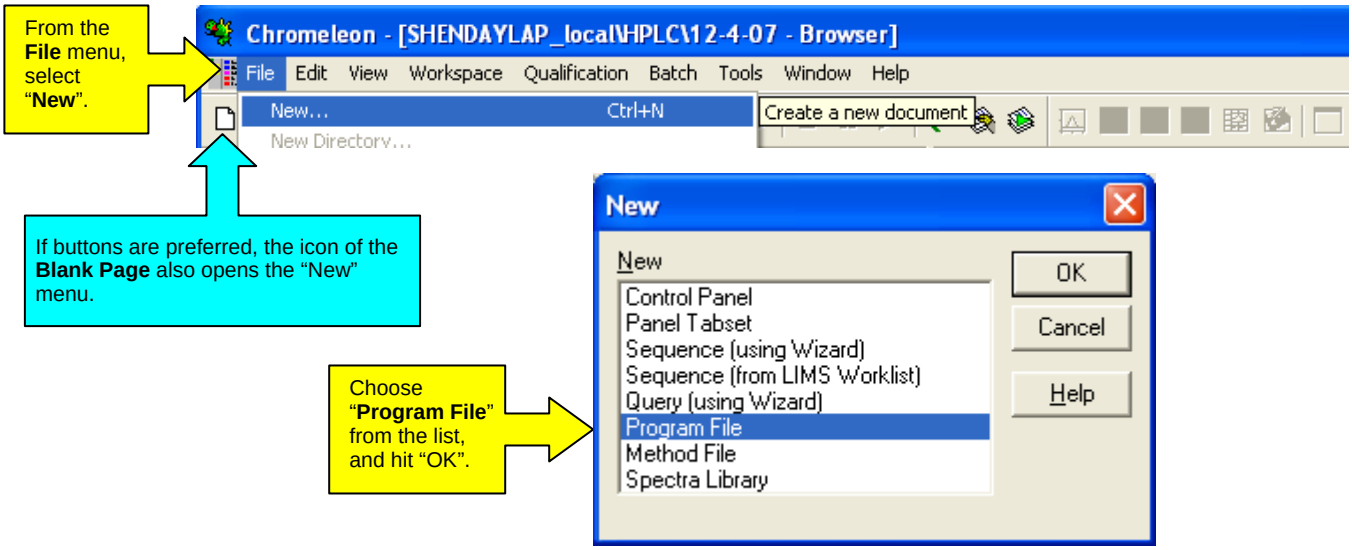

(12) Save the tabset with the added MSQ panel.
IC_MS
Sample Information
Timebase: IC_MS
Datasource:
Sequence:
Current Sample:
Type: n/a
Retention Time:
Sample No.:
Sample End:
MS
Connected
Ready to Download
Range - Online:
Operation:
Smoothing:
Smoothing Points:
Audittrail
Mass Spectrum

3.5 Program Files

Program files define the hardware parameters for the chromatographic separation and detection. The samples are run with program files in order to separate the individual analytes of the sample mixture.

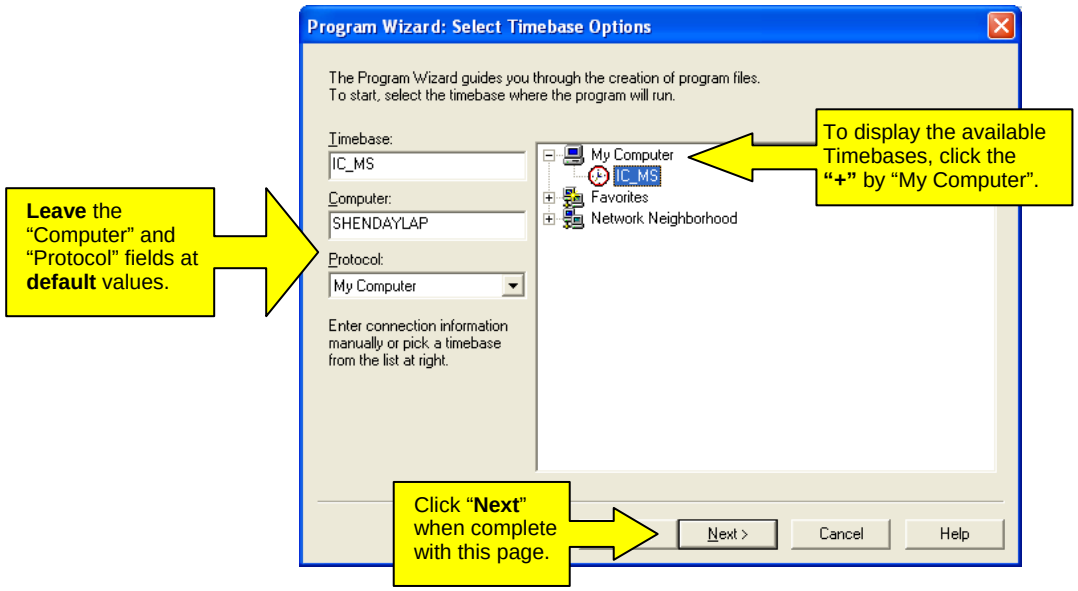
To create a program, start from either the Panel Tabset or the Browser page:

1. Go to the File menu.
2. Select "New…" to create a new document.

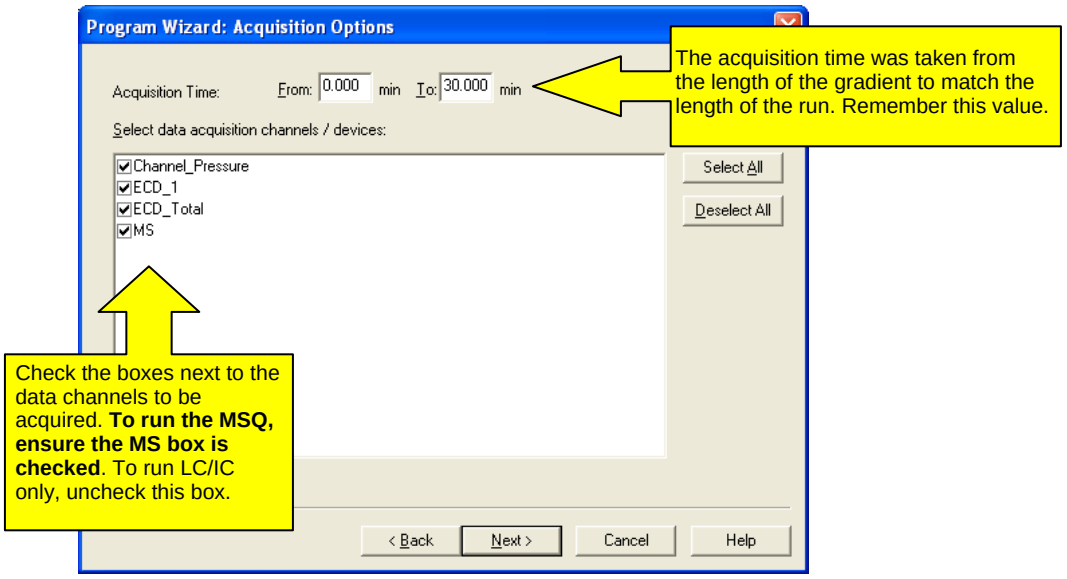


3. A menu appears, select "Program File".

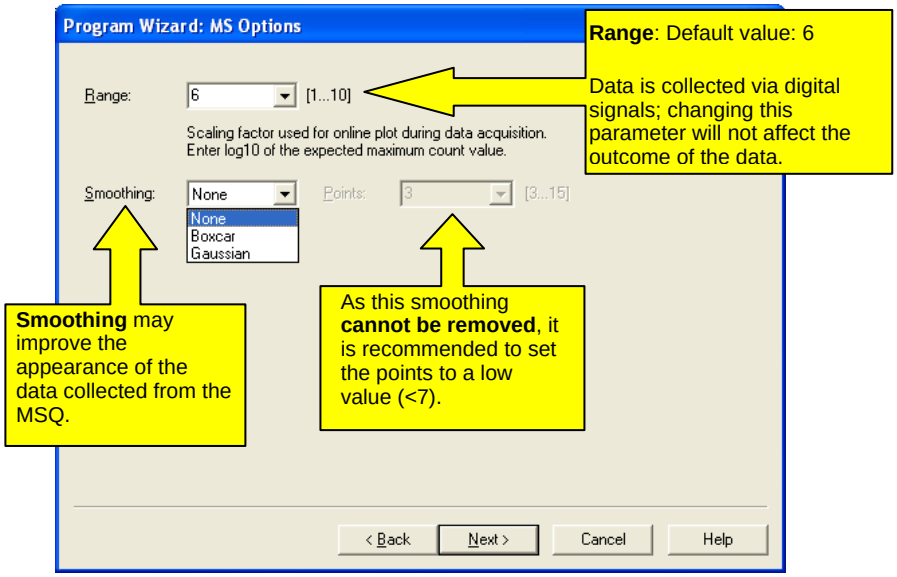
4. The Program Wizard automatically opens. The first question requests the "Timebase". A timebase is a group of hardware modules that work in concert; you may be more familiar with the definition "system" or "pump, detector, oven, etc.".


Program Wizard: Select Timebase Options
The Program Wizard guides you through the creation of program files.
To start, select the timebase where the program will run.
Timebase:
IC_MS
Computer:
SHENDAYLAP
Protocol:
My Computer
Enter connection information manually or pick a timebase from the list at right.
My Computer
IC_MS
Favorites
Network Neighborhood
To display the available Timebases, click the "**+**" by "My Computer".
Leave the "Computer" and "Protocol" fields at **default** values.
Click "**Next**" when complete with this page.
Next >
Cancel
Help

5. Click through the pages of the wizard to define all parameters of the system (*e.g.*, gradient, oven temperatures, etc.). These values will be unique per application.
6. Continue to complete the wizard settings for your application. When you reach the Acquisition Options of the wizard, pay close attention:
    A. **Acquisition Time**: Information gathered from the length of your gradient. This field is customizable for the length of time you would like the data collected.
    B. **Channels**: Check the boxes next to the signals for which you would like to collect data.
        1. If you would like to collect MS data, the box next to MS must be checked.
        2. If you would not like to collect MS data for this run (IC/LC only), uncheck this box.


Program Wizard: Acquisition Options
Acquisition Time:
From: 0.000 min
To: 30.000 min
Select data acquisition channels / devices:
Channel_Pressure
ECD_1
ECD_Total
MS
Select All
Deselect All
< Back
Next >
Cancel
Help
The acquisition time was taken from the length of the gradient to match the length of the run. Remember this value.
Check the boxes next to the data channels to be acquired. To run the MSQ, ensure the MS box is checked. To run LC/IC only, uncheck this box.

7. Continue to follow the steps of the wizard to program the IC/LC detector.
8. Several steps later, the wizard will ask for options on the mass spectrometer.
    A. **Range:** Sets the scale of the signal to be plotted by the MSQ in Chromeleon.
    B. **Smoothing**: Improves the signal noise, chromatogram appearance, and reproducibility of peak baselines by applying different digital filters to the raw data. Once applied, these filters cannot be removed. To maintain the integrity of the data, it is recommended to set the points to a low number. (e.g., <7 Points). If more smoothing is required, filters can be added later, during the data quantification.
        1. **None**: No smoothing will be applied.
        2. **Boxcar**: Synonymous with “Moving Average” filter; data points are equally weighted. As such, its ability to discriminate between noise and signal is limited
        3. **Gaussian**: Applies the Gaussian distribution for chromatogram smoothing.

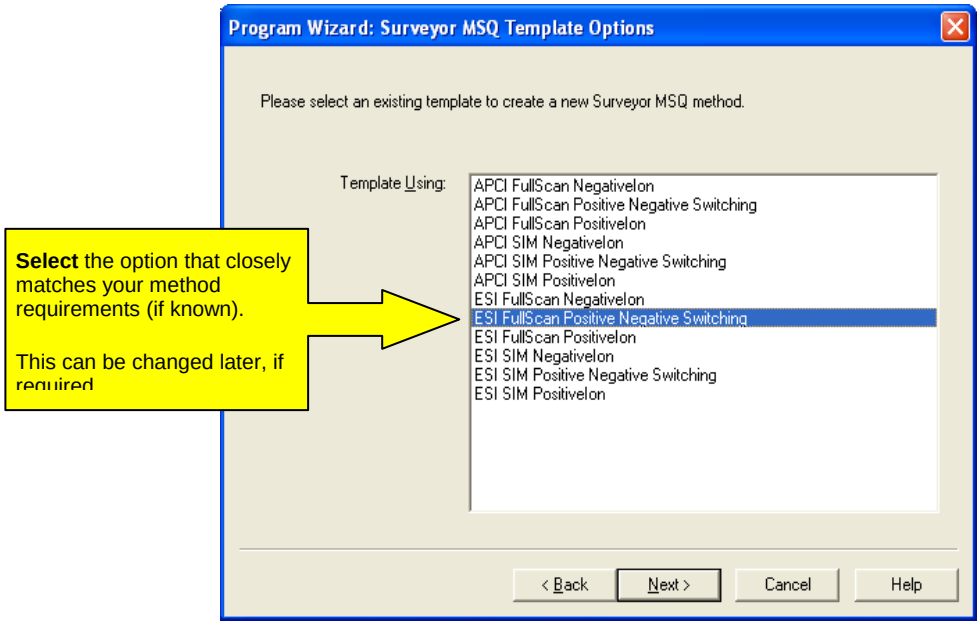

Program Wizard: MS Options
Range: 6 [1...10]
Scaling factor used for online plot during data acquisition.
Enter log10 of the expected maximum count value.
Smoothing: None
None
Boxcar
Gaussian
Points: 3 [3...15]
< Back
Next >
Cancel
Help
Range: Default value: 6
Data is collected via digital signals; changing this parameter will not affect the outcome of the data.
Smoothing may improve the appearance of the data collected from the MSQ.
As this smoothing cannot be removed, it is recommended to set the points to a low value (<7).

9. Click Next to define the scan conditions for the MSQ Plus.
10. The wizard creates a scan template based upon the selection in this page.

Probes

A. **APCI**: Atmospheric Pressure Chemical Ionization. Typically used on nonpolar compounds <1000 Da.

B. **ESI**: Electrospray Ionization. Typically used on polar compounds, always used in ion chromatography (IC) applications.


Program Wizard: Surveyor MSQ Template Options
Please select an existing template to create a new Surveyor MSQ method.
Template Using:
APCI FullScan Negativeion
APCI FullScan Positive Negative Switching
APCI FullScan Positiveion
APCI SIM Negativeion
APCI SIM Positive Negative Switching
APCI SIM Positiveion
ESI FullScan Negativeion
ESI FullScan Positive Negative Switching
ESI FullScan Positiveion
ESI SIM Negativeion
ESI SIM Positive Negative Switching
ESI SIM Positiveion
< Back
Next >
Cancel
Help
Select the option that closely matches your method requirements (if known).
This can be changed later, if required

Scan Type

A. **FullScan**: Scans across a definable *mass-to-charge (m/z)* range.

   1. This option is selected to provide the most information about the sample. (*i.e.*, A mass spectrum can be generated from a full scan.) Can be used as a screening tool if *m/z* is unknown.

   2. The least sensitive mode for quantification.

B. **SIM**: Selected Ion Monitoring. The data collected by the mass spectrometer is focused on a single mass, with a narrow window.

   1. This option is selected to provide the most sensitivity (high signal: noise ratio) for the analytes.

   2. The *m/z* must be known prior to selecting SIM as a scan type.

Polarity

A. **Positive**: Data is only collected for cationic species (positively charged analytes).

B. **Negative**: Data is only collected for anionic species (negatively charged analytes).

C. **Positive Negative Switching**: The MSQ will alternate scans between positive and negative ions allowing for both cationic and anionic species to be detected in a single run.

   1. Can be used as a tool for screening if the polarity of a sample is unknown.

Relay and State Devices Options

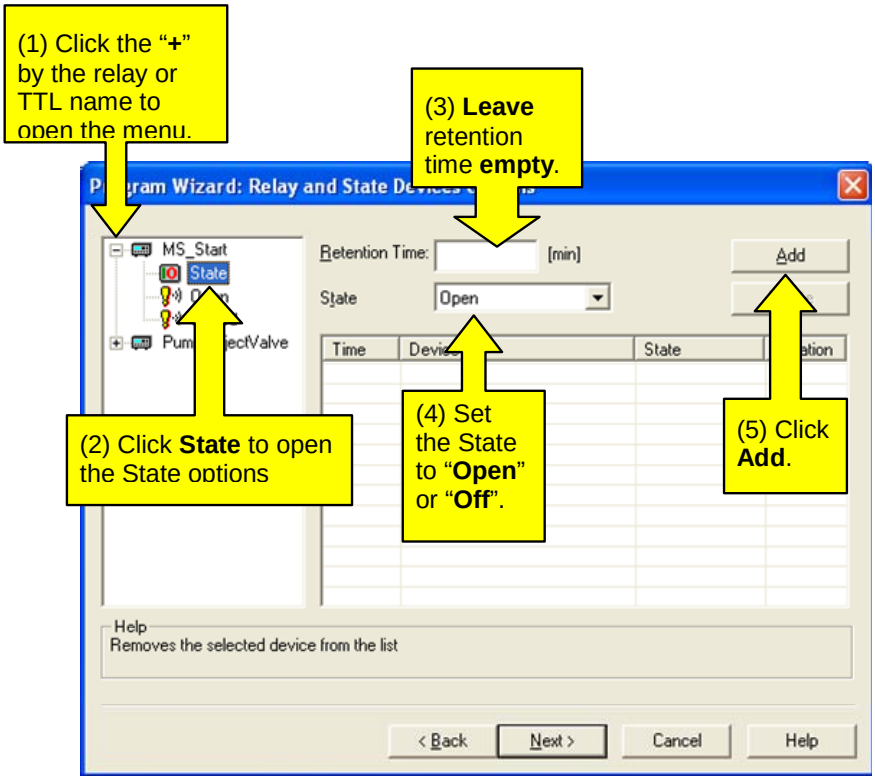
Click Next to enter the "Relay and State Devices Options" page. In order to trigger the mass spectrometer to start collecting data, a relay closure is given from the IC or LC device. Upon installation of the system, the cabling was connected. In the program, the steps to trigger the device must be added to the program file.

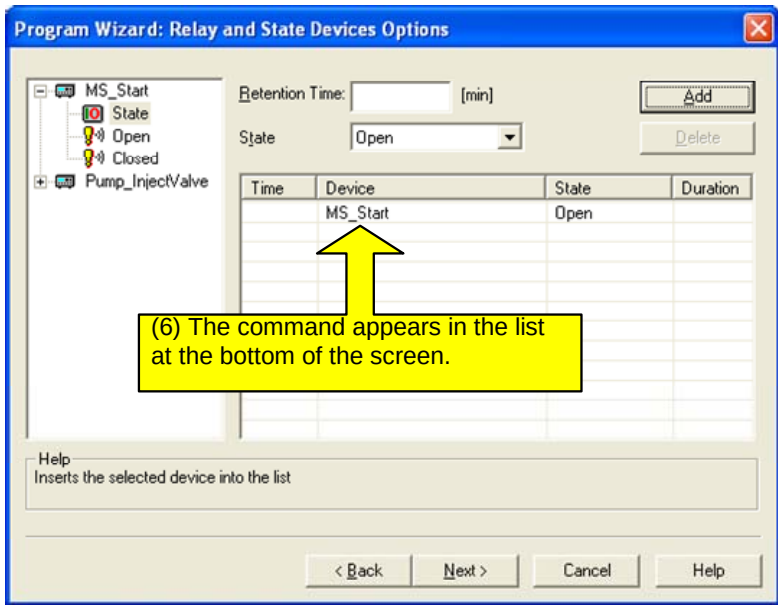
A. It is recommended to always have the TTL or Relay in the Open position at the start of the program. To trigger this:

   1. Click on the "+" by the TTL/Relay name. (In this example, the TTL was named "MS_Start")
   2. Click "State".
   3. Leave the Retention Time blank.
   4. Select Open or Off from the "State" Options.
   5. Click "Add".

B. The command to start the mass spectrometer must also be programmed.

1. Click on the “Closed” or “On” option under the relay name.
2. In the retention time field, enter the time for the mass spectrometer to start acquiring data.





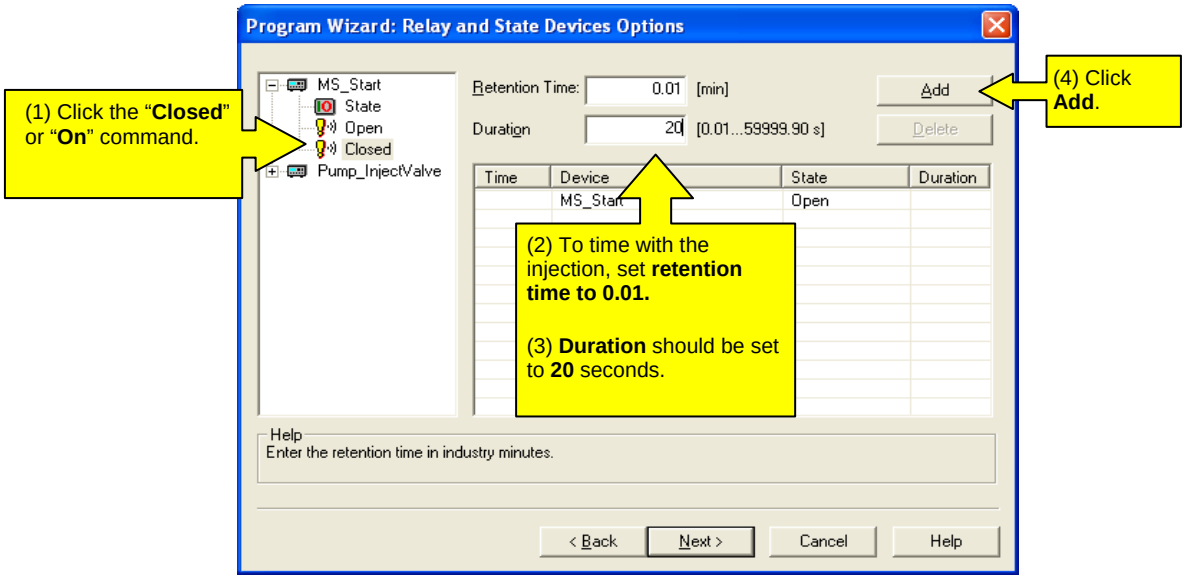
What commands will you need to add?

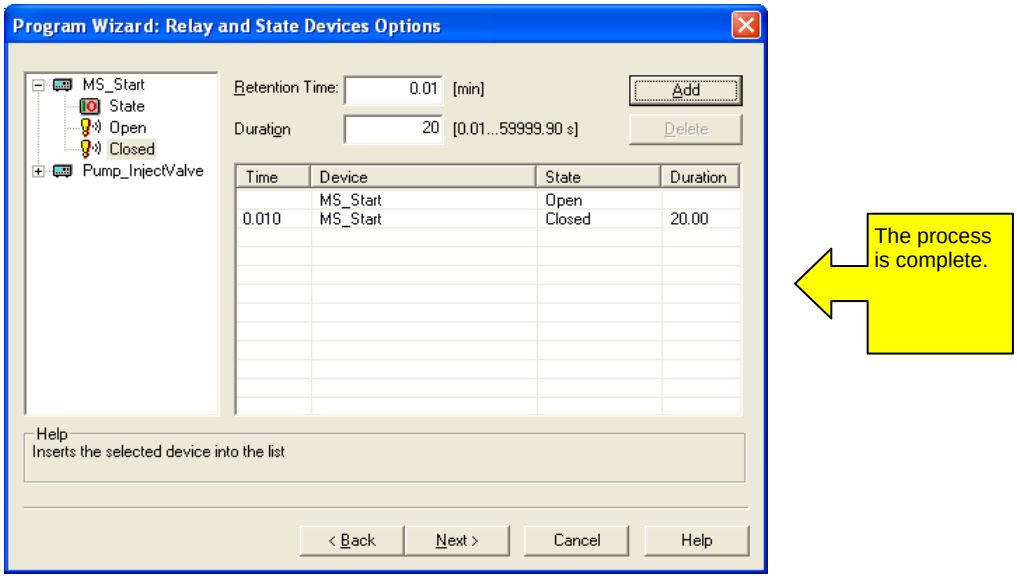
Use this space to make a list of the relay commands you will need in order to control your system (*e.g.* matrix diversion valves, external water for IC systems)

a. If the first few minutes of a run are not important, enter the delay time here.

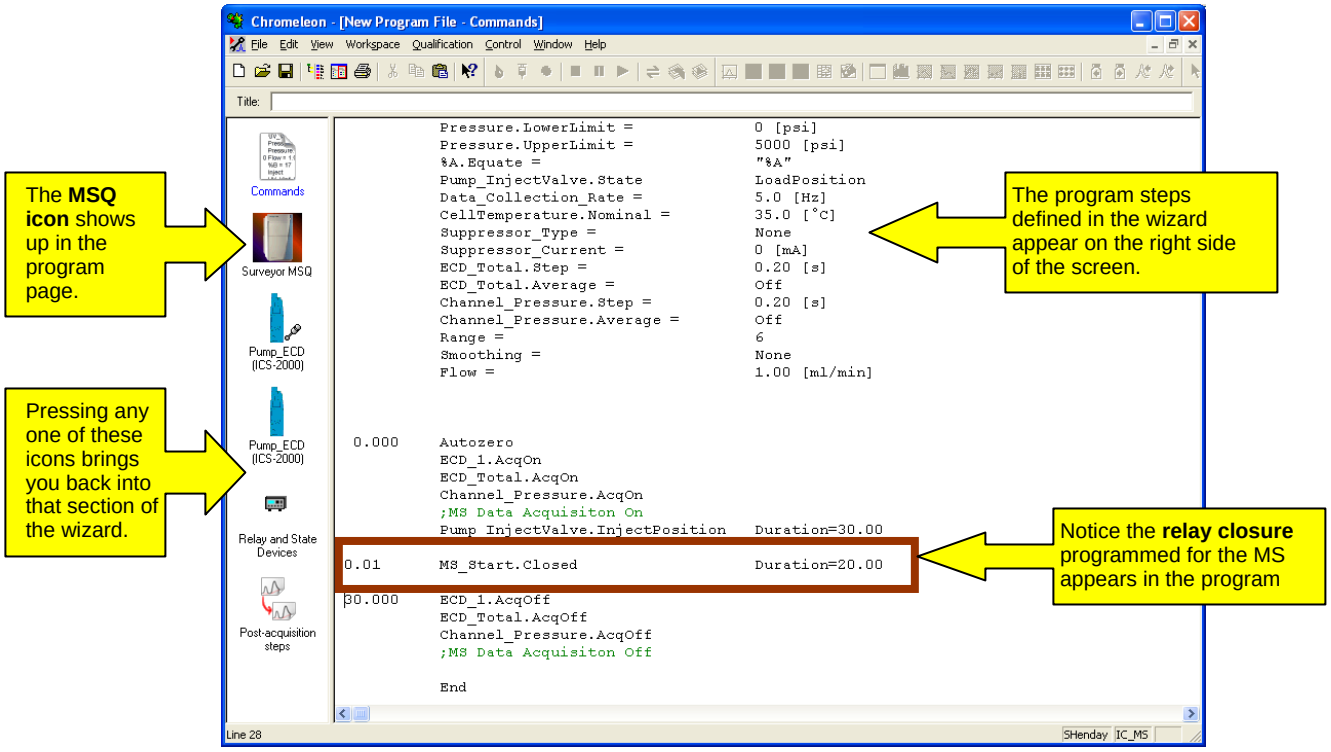
b. If the run is to be synchronized with the inject command, enter "0.01" [minutes] into the blank.

3. Set the duration to "20" seconds.
4. Click "Add".





Upon completion of the Program Wizard, the program information is displayed in a basic format.



Mass Spec Scan Parameters

Click the icon for the mass spectrometer to define the scan parameters of the program file.

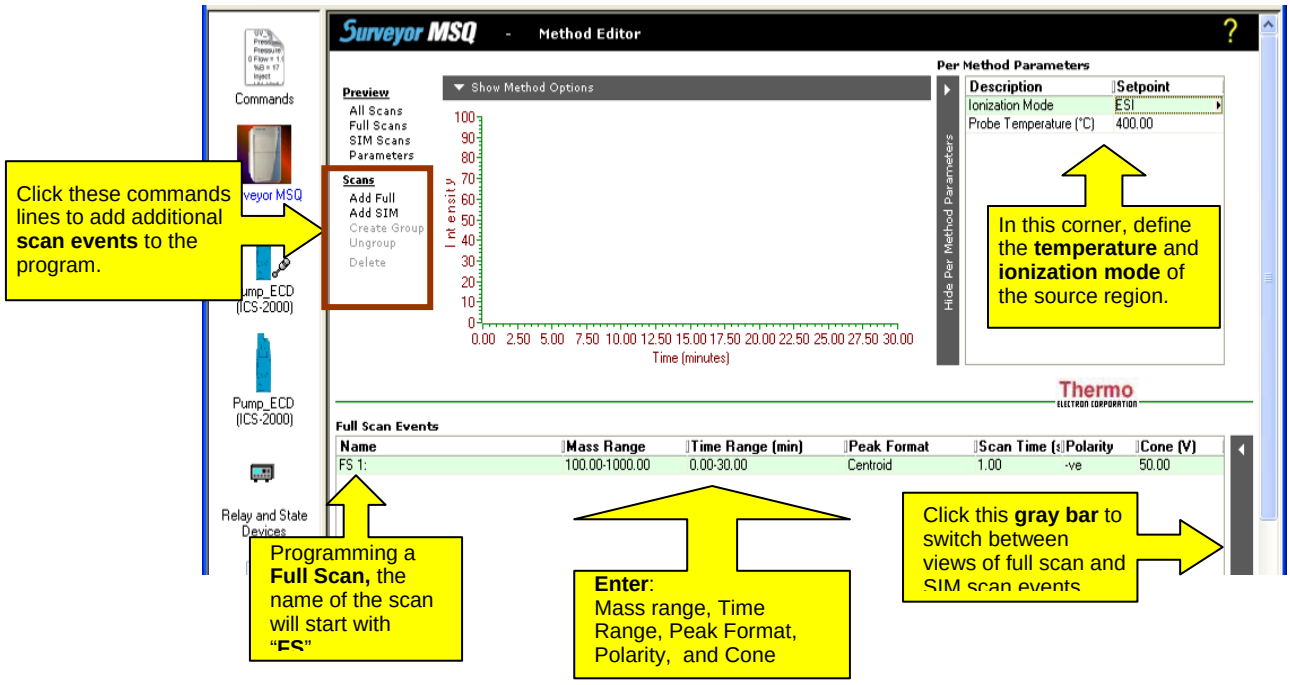
A. In the upper right section of the screen, you will see the field for ionization mode (ESI or APCI) and **Probe Temperature**.

   1. The probe temperature is the temperature in degrees Celsius that the nitrogen gas is heated to by the Probe to desolvate your sample.
   2. There are several factors that will affect the probe temperature:
      a. Solvent composition
      b. Flow rate
      c. The thermal labile properties of your sample
   3. The probe temperature will have to be optimized for each application.

B. In the lower portion of the screen, define the scan functions.

   1. **Name**: “FS1” in the name field indicates the parameter fields are for a full scan event.

2. **Mass Range**: Enter the range of atomic mass units (amu) for the detector to scan. The software for the MSQ Plus is calibrated to scan from 23–1972 amu.
3. **Time Range**: Define the time (in minutes) for the run to be collecting the data.
4. **Peak Format**: Sets the scan function for how data is collected.
    a. Centroid: The most common way to collect full scan spectra. Nearby masses are averaged into a shape resembling “sticks”.
    b. Profile: Collects data that visually resembles chromatographic peaks. Yields a great deal of information about the mass of your specific analyte, but takes a lot of memory on the computer (10x more hard drive space is required when using Profile mode over Centroid mode.)

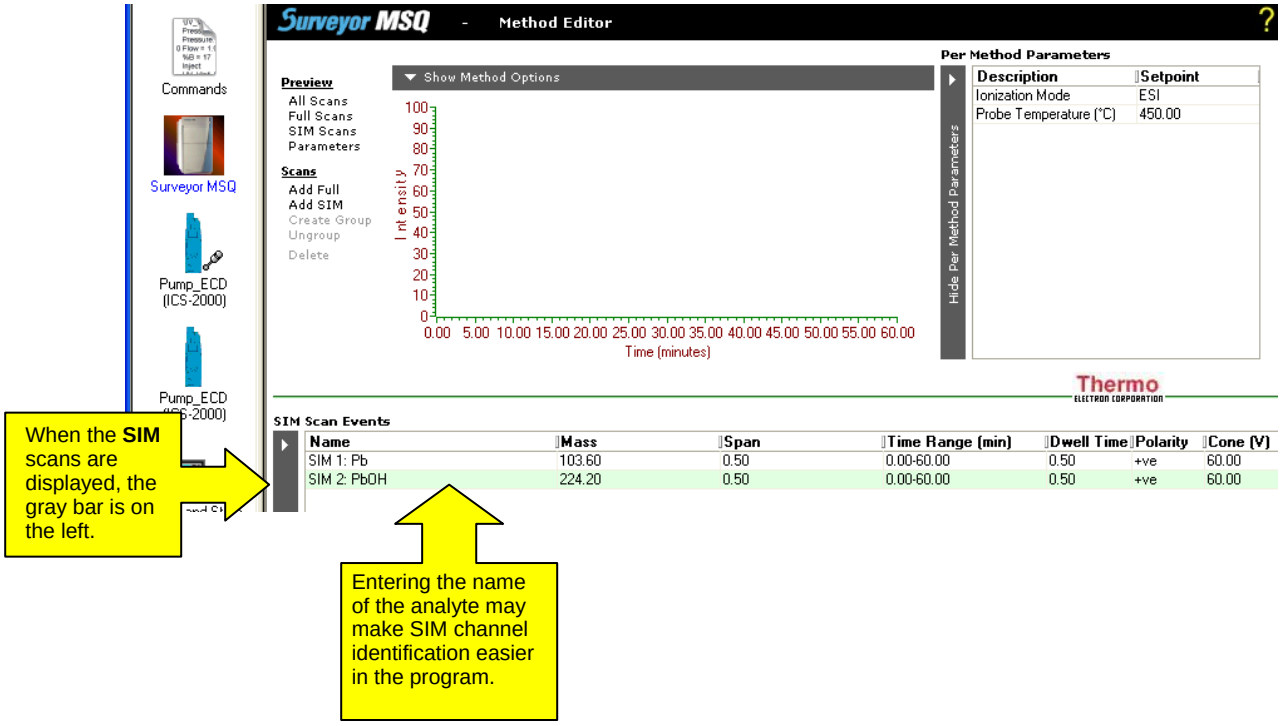


5. **Scan Time**: The amount of time you give the detector to scan across the mass range. Scanning within the range of 250–1000 amu/second is ideal when using Full Scan mode).
6. **Polarity**: Positive or Negative. If you would like to scan both positive and negative polarities, add another full scan event with the opposite polarity.
7. **Cone Voltage**: The amount of energy applied to the source region of the mass spectrometer. Attracts the ion beam into the analyzer, but excess energy can cause the molecule to break apart, or fragment. [Can cause Collision Induced Dissociations (CID fragments) in this region to help identify unknowns.]

C. To add another full scan event, locate the “Add Full” command in the middle of the page. Pressing this command line adds another full scan event to the list.

D. To switch between the views of SIM and full scan events, locate the grey bar at the bottom of the screen.

1. In full scan mode, the bar is on the right side of the screen.
2. In SIM mode, it is on the left side of the screen.
3. Click on this bar. As the bar wipes across the screen, it changes the view from full to SIM scan events.



E. Programming SIM scan events is similar to programming full scan events.

1. The **probe temperature** and **ionization mode** are global parameters, they do not need to be changed for SIM scans.
2. **Name**: SIM 1: In this field, you can also add the name or chemical formula of your analyte.
3. **Mass**: Enter the single mass you are interested in scanning.
4. **Span**: The mass window around the desired mass.
   a. Value is total window value. (*e.g.*, Setting the *m/z*: 195 span: 0.30 → scans *m/z* 194.85-195.15.)
   b. Lower span values generally offer more sensitivity, but may be less robust. A slight shift in mass could cause the scans to be on the peak shoulder, not centered on the apex.

5. **Time Range**: Enter the time range (in minutes) to scan for this mass in your chromatographic run.
    a. When running several scan functions, consider specific time ranges for each SIM channel.
    b. Staggering the time range for each peak minimizes the total analytical cycle time and optimizes the signal to noise.
6. **Dwell Time:** The time spent (in seconds) on scanning this specific mass. Dwell time is critical for optimizing signal-to-noise on the peak.
    a. This parameter must be optimized for each application.
    b. Its value also depends on how many channels of data you are acquiring from the mass spectrometer. Be wary with total analytical cycle time with respect to dwell time.
    c. Calculate to measure ***at least*** 10 measurements across the chromatographic peak.
7. **Polarity**: Positive or negative polarity (anionic or cationic species).
8. **Cone Voltage**: Analyte specific, this value is user optimized to determine maximum peak sensitivity and the desired CID.

3.6 Shutdown Programs

At the end of the run, the system should be placed into "standby mode". In this mode, the eluent flow is either turned off or diverted away from the mass spectrometer. When the mass spectrometer enters standby mode, the high voltages, heater, and nitrogen gas flow turn off.

Shutdown Parameters

Depending on the configuration, the hardware and software requirements to place the system into standby mode will differ. Things to remember to turn off on the front end are:

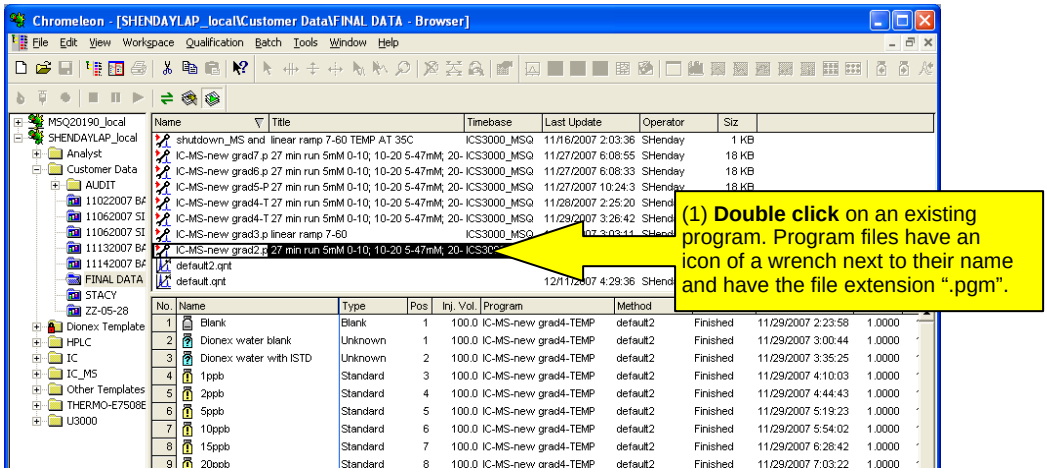
- Flow to the column
- UV lamp
- Ovens
- Eluent generator, suppressors, CR-TCs (for IC customers)

The easiest way to create a shutdown program is to modify an existing program to include the shutdown parameters, and save this file under a different name.

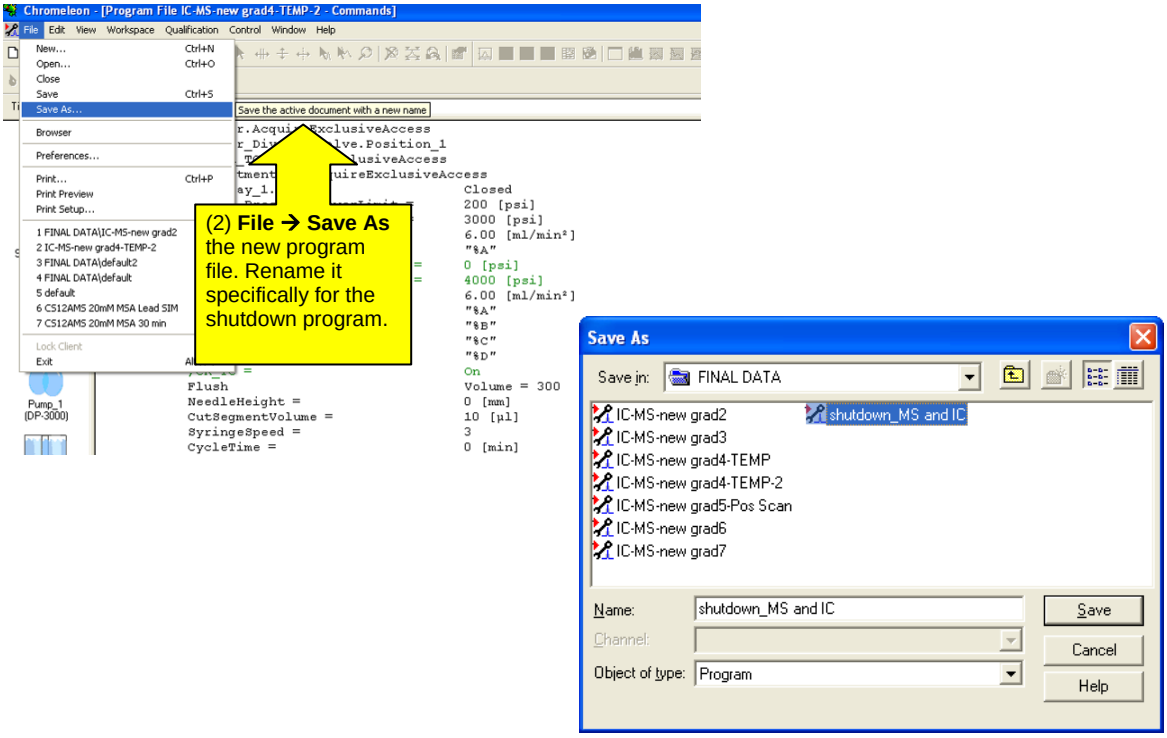
Creating a Shutdown Program for the Mass Spectrometer

While it is difficult to create a specific example of a shutdown program that will apply to all customers, the following steps are required to shutdown the mass spectrometer at the end of the sequence:

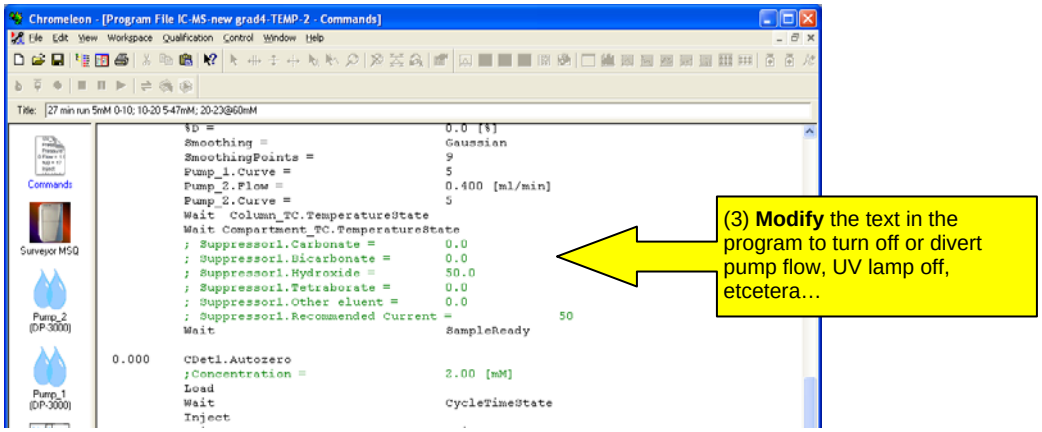
1. Open an existing program by double clicking on the file name. In this example, open the program file associated with the sequence.


Chromeleon - [SHENDAYLAP_local\Customer Data\FINAL DATA - Browser]
(1) **Double click** on an existing program. Program files have an icon of a wrench next to their name and have the file extension ".pgm".

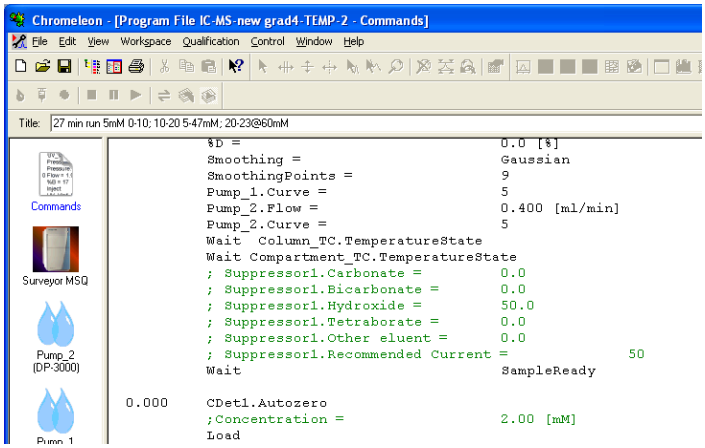
2. File → Save As the program to a new name, such as "Shutdown Method".



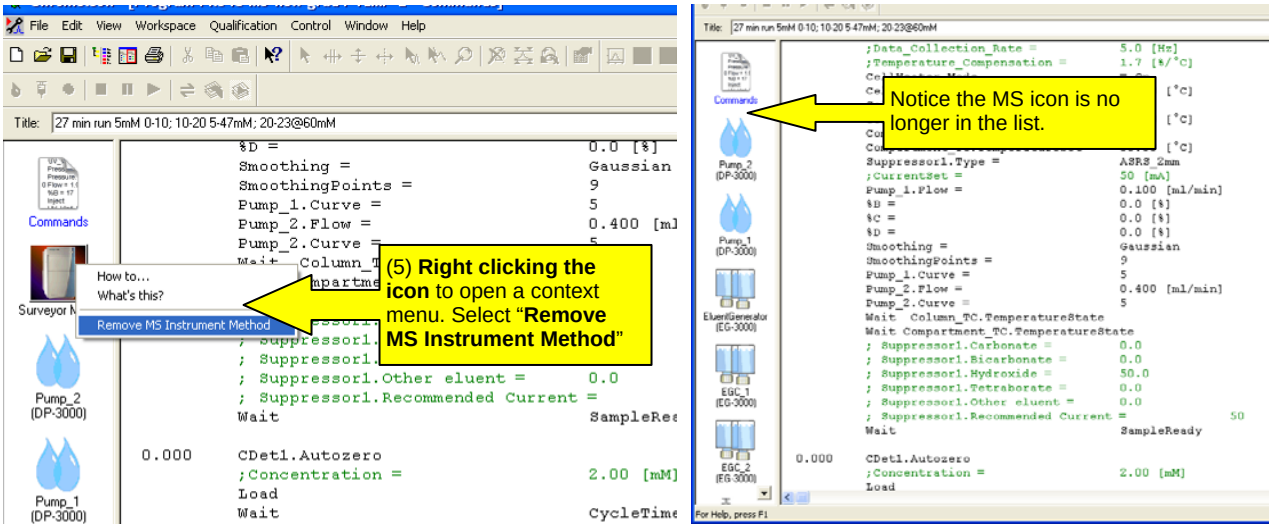
3. Modify the IC/LC parameters to turn off the flow or divert the flow away from the mass spectrometer.


Chromeleon - [Program File IC-MS-new grad4-TEMP-2 - Commands]
(3) **Modify** the text in the program to turn off or divert pump flow, UV lamp off, etcetera…

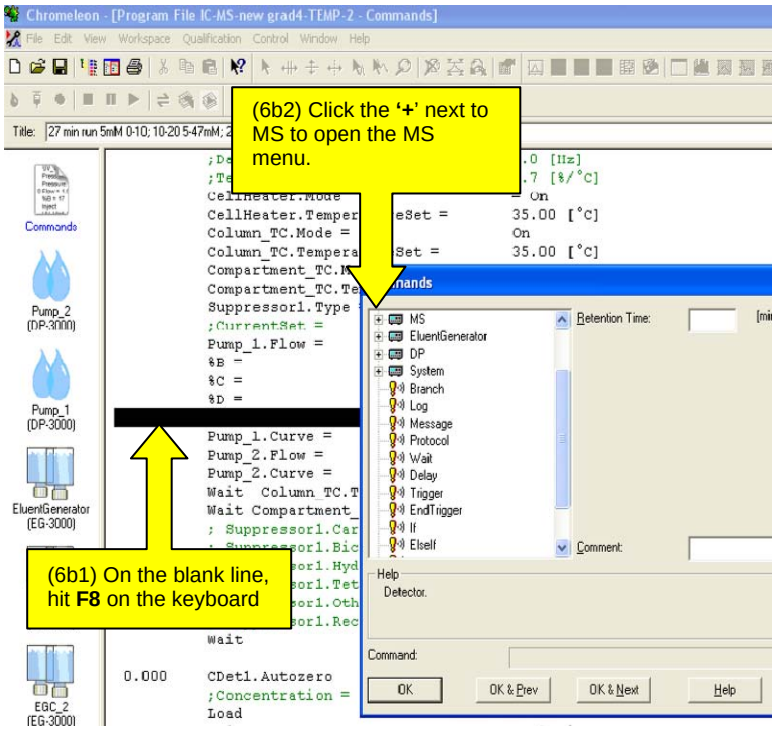
4. Right Click on the icon for the mass spectrometer in the method.



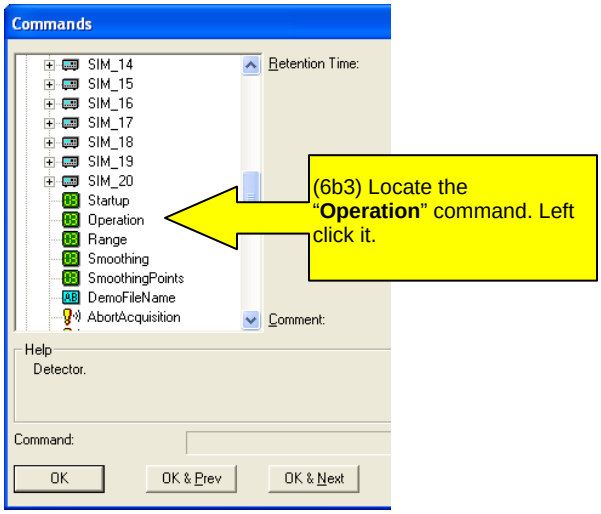
5. Select “Remove MS Instrument Method” from the context menu.


How to...
What's this?
Remove MS Instrument Method
(5) **Right clicking the icon** to open a context menu. Select “**Remove MS Instrument Method**”
Notice the MS icon is no longer in the list.

6. Replace the commands for smoothing, with the command line “Operation = StandbyNoWarning”. To add this command:
    a. Manually type in “Operation = StandbyNoWarning”
    b. Via the commands menu:
        i. Press the F8 button on the keyboard to open the commands menu.

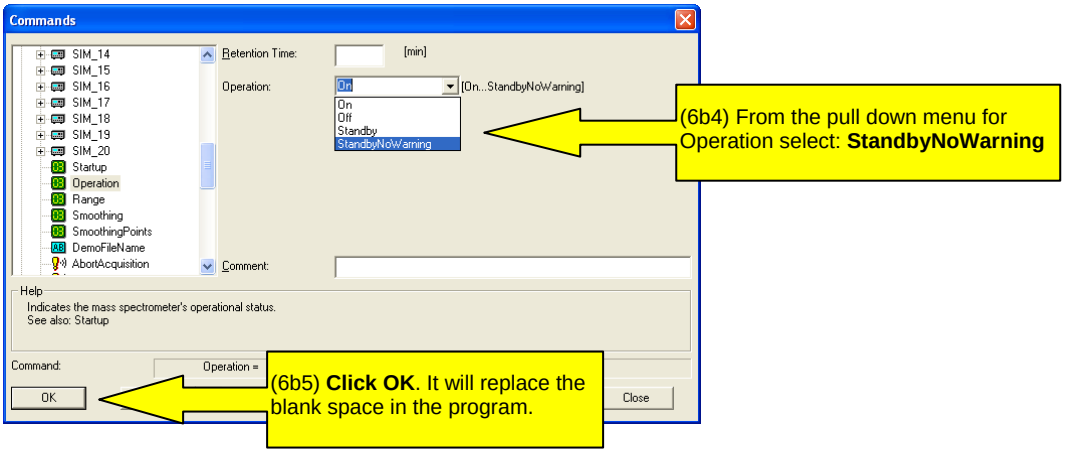


        ii. Click the “+” by “MS”
        iii. Scroll down the MS list to find a green icon next to the word “Operation”. Click on the word “Operation”.



iv. From the pull down list on the right for Operation, select "StandbyNoWarning".

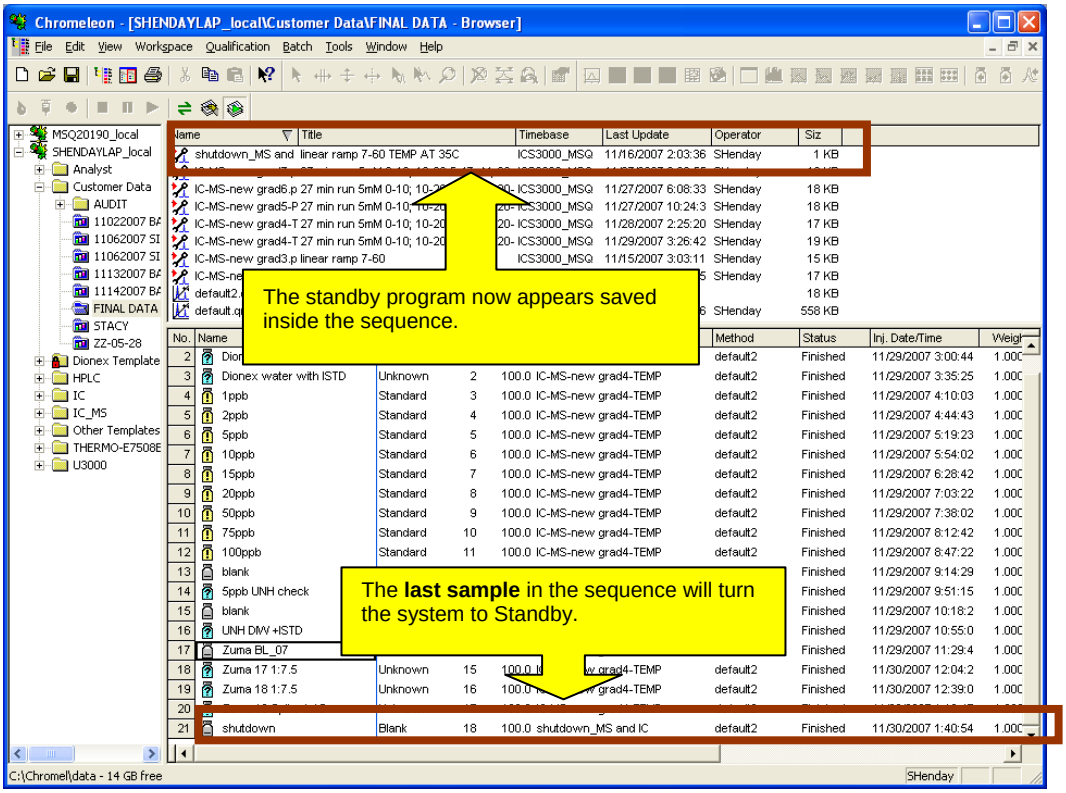
v. Click OK to add this command to the program.



7. Save the program file.

8. Add a sample to the end of your sequence with the following parameters:

   - Name = Shutdown
   - Type = Blank
   - Inj. Volume = (make sure this is specific for your system).
   - Program File: Select the shutdown/standby program file you just created.



3.7 Sequences

Once the program file is created, a sequence is built. The sequence has two functions:

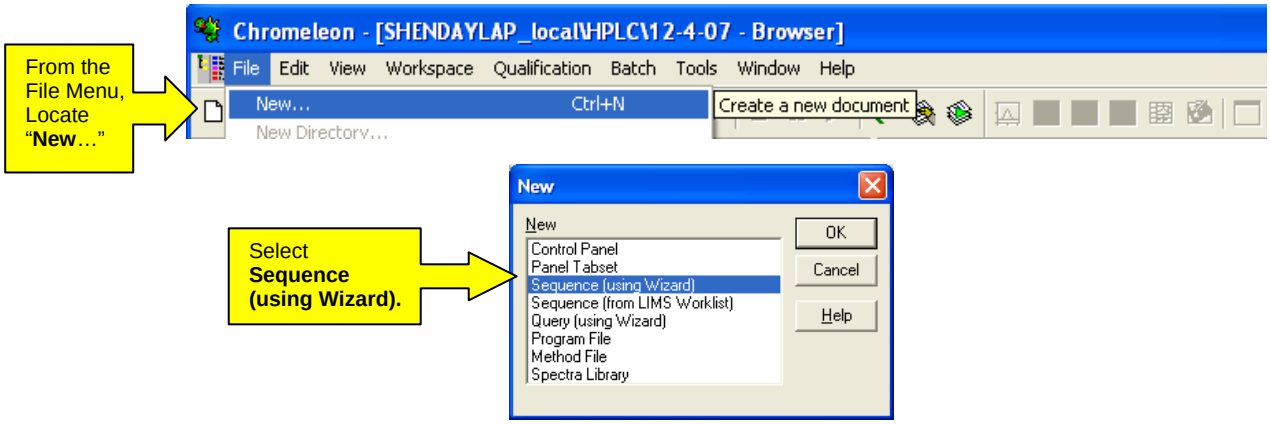
1. **Sequences organize the samples** in the order to be analyzed by the instrument.
2. **The sequence stores the data collected** from the analysis.

There are two avenues to create a sequence:

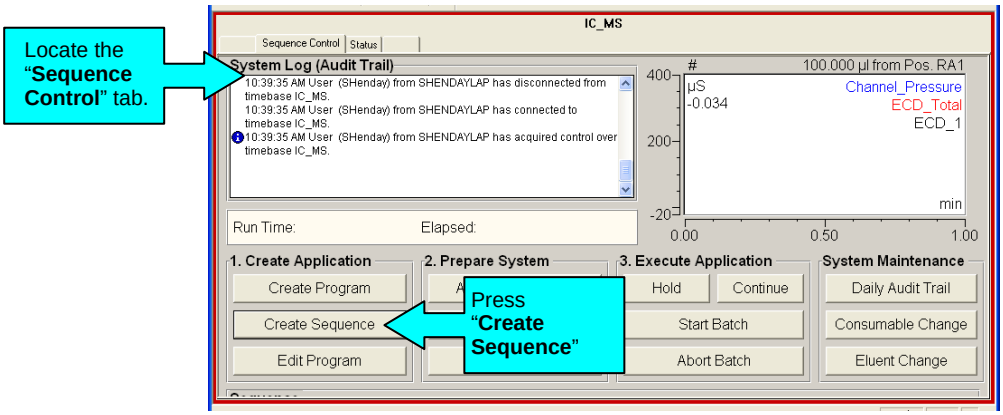
1. Sequence wizard
2. Modification of an existing sequence

Chromeleon Sequence Wizard

A. From the File Menu, select "New…". From the "New" Menu, select "Sequence (using Wizard)".



B. **Alternatively**, access the sequence wizard by pressing the "Create Sequence" button on the Panel Tabset "Sequence Control" page. Both modes lead to the wizard.



C. Follow the five steps of the sequence wizard to create the sequence shell. (Use the technique you learned from the Program Wizard.)

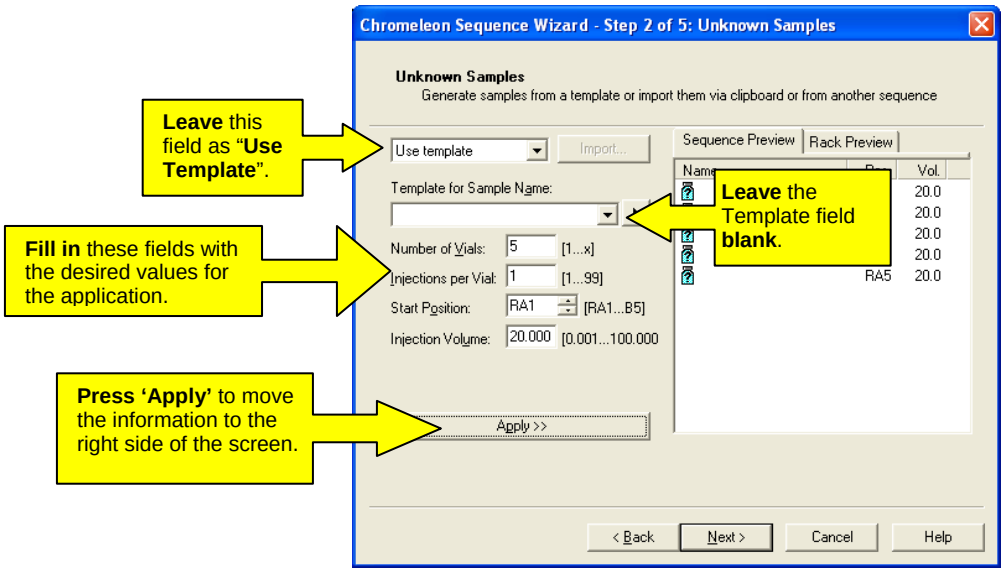
a. Identify the timebase

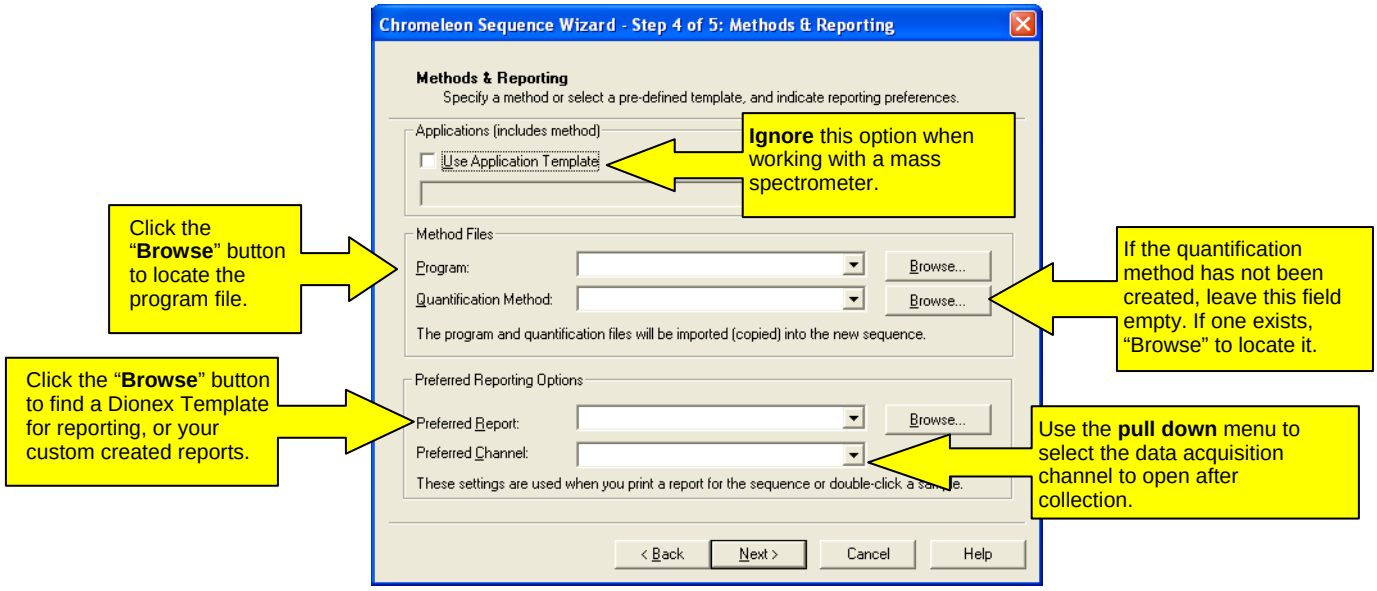
b. Define unknown sample parameters

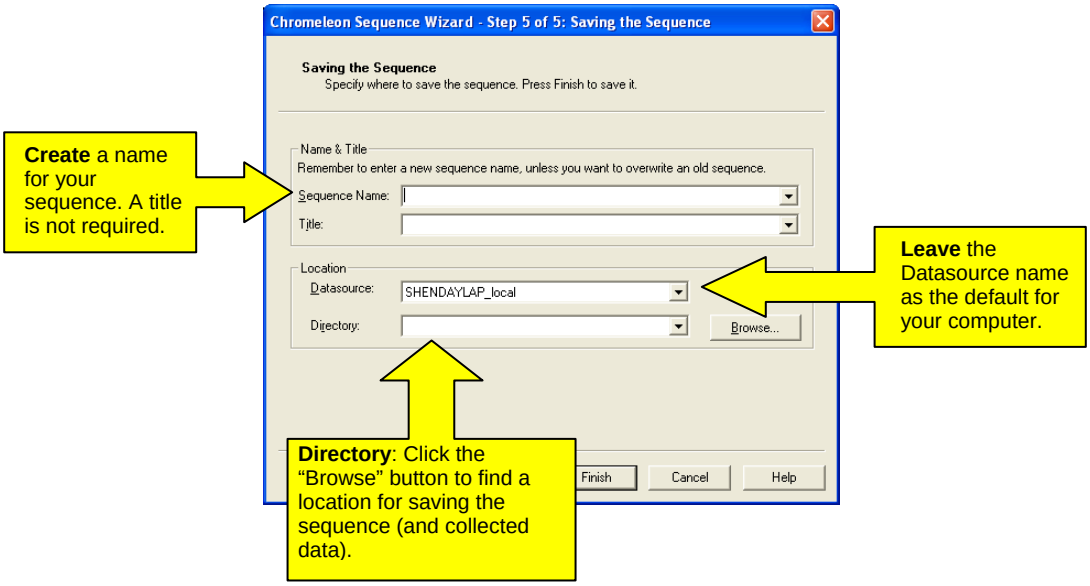
c. Define parameters for the standards

d. Define method and reporting options for the sequence

e. Identify where to store the sequence and data collected


Chromeleon Sequence Wizard - Step 2 of 5: Unknown Samples
Unknown Samples
Generate samples from a template or import them via clipboard or from another sequence
Use template
Import...
Template for Sample Name:
Number of Vials: 5 [1...x]
Injections per Vial: 1 [1...99]
Start Position: RA1 [RA1...B5]
Injection Volume: 20.000 [0.001...100.000
Apply >>
Sequence Preview
Rack Preview
Vol.
20.0
RA5
< Back
Next >
Cancel
Help
Leave this field as "Use Template".
Leave the Template field blank.
Fill in these fields with the desired values for the application.
Press 'Apply' to move the information to the right side of the screen.

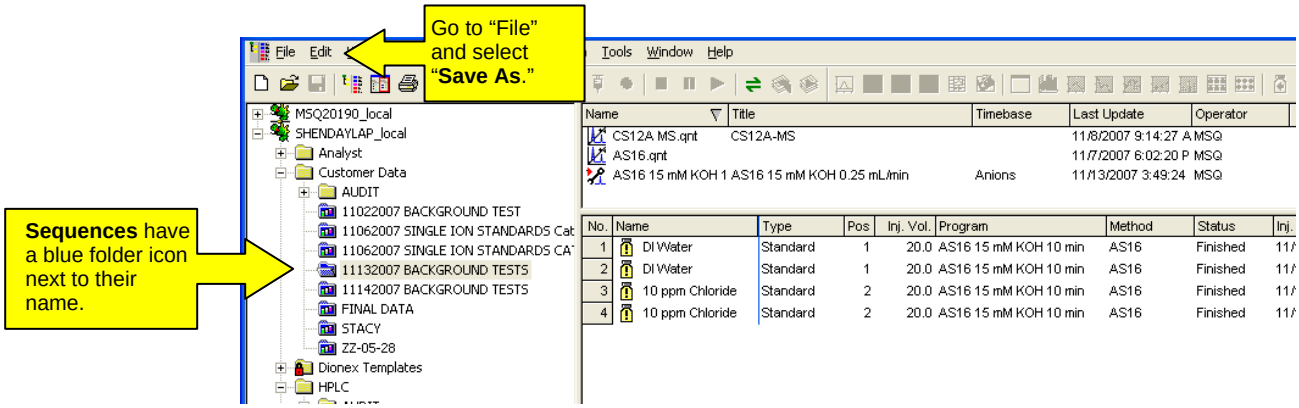

Chromeleon Sequence Wizard - Step 4 of 5: Methods & Reporting
Methods & Reporting
Specify a method or select a pre-defined template, and indicate reporting preferences.
Applications (includes method)
Use Application Template
Method Files
Program:
Quantification Method:
Browse...
The program and quantification files will be imported (copied) into the new sequence.
Preferred Reporting Options
Preferred Report:
Preferred Channel:
These settings are used when you print a report for the sequence or double-click a sample.
< Back
Next >
Cancel
Help
Ignore this option when working with a mass spectrometer.
Click the "Browse" button to locate the program file.
If the quantification method has not been created, leave this field empty. If one exists, "Browse" to locate it.
Click the "Browse" button to find a Dionex Template for reporting, or your custom created reports.
Use the pull down menu to select the data acquisition channel to open after collection.


Chromeleon Sequence Wizard - Step 5 of 5: Saving the Sequence
Saving the Sequence
Specify where to save the sequence. Press Finish to save it.
Name & Title
Remember to enter a new sequence name, unless you want to overwrite an old sequence.
Sequence Name:
Title:
Location
Datasource:
SHENDAYLAP_local
Directory:
Browse...
Finish
Cancel
Help
Create a name for your sequence. A title is not required.
Leave the Datasource name as the default for your computer.
Directory: Click the "Browse" button to find a location for saving the sequence (and collected data).

Modifying an Existing Sequence

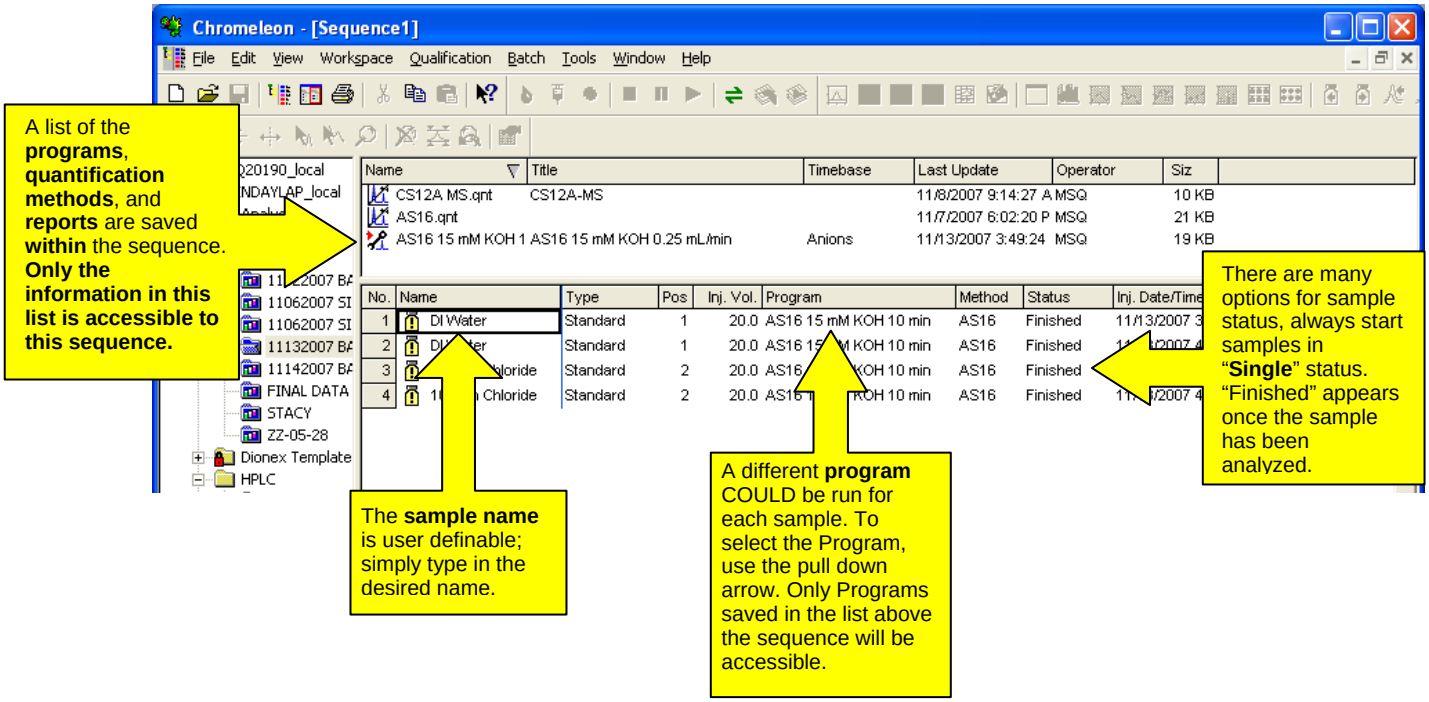
In order to modify an existing sequence, open the previous sequence in the Browser. ***Recall: Sequences are identified by the blue folder icon next to their names.***

1. Open an existing sequence.
2. From the File Menu select "Save As".
3. Specify a new name for the sequence about to be created.
4. Click Save.



5. A new sequence is created. All sample information (**not data**) from the old sequence is found in the new sequence, therefore the calibration data files are not carried forward. If you do not wish to run a new calibration curve for the day, the Finished runs for the Standards would need to be copied from the old sequence and pasted into the new sequence.
6. Rename the samples, position in the autosampler, and injection volume as relevant.

3.8 Familiarization with the Sequence Format


Chromeleon - [Sequence1]
File Edit View Workspace Qualification Batch Tools Window Help
Name Title Timebase Last Update Operator Siz
CS12A MS.qnt CS12A-MS 11/8/2007 9:14:27 A MSQ 10 KB
AS16.qnt 11/7/2007 6:02:20 P MSQ 21 KB
AS16 15 mM KOH 1 AS16 15 mM KOH 0.25 mL/min Anions 11/13/2007 3:49:24 MSQ 19 KB
No. Name Type Pos Inj. Vol. Program Method Status Inj. Date/Time
1 DI Water Standard 1 20.0 AS16 15 mM KOH 10 min AS16 Finished 11/13/2007 3
2 Standard 1 20.0 AS16 Finished
3 Standard 2 20.0 AS16 Finished
4 Standard 2 20.0 AS16 Finished
FINAL DATA
STACY
ZZ-05-28
Dionex Template
HPLC

A list of the **programs**, **quantification methods**, and **reports** are saved **within** the sequence. **Only the information in this list is accessible to this sequence.**

There are many options for sample status, always start samples in "**Single**" status. "Finished" appears once the sample has been analyzed.

The **sample name** is user definable; simply type in the desired name.

A different **program** COULD be run for each sample. To select the Program, use the pull down arrow. Only Programs saved in the list above the sequence will be accessible.

NOTE: NEVER CHANGE THE STATUS OF A SAMPLE FROM 'FINISHED' TO 'SINGLE' WHEN OPERATING A MASS SPECTROMETER. ALWAYS ADD A NEW SAMPLE TO THE LIST.

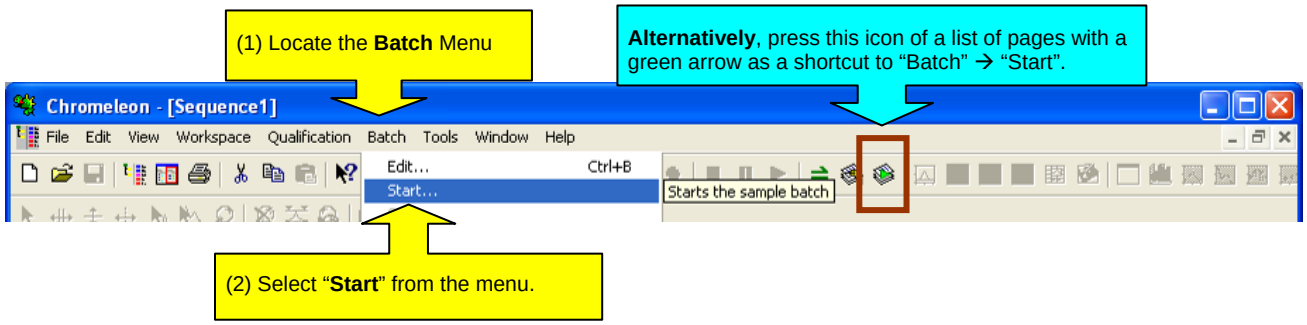
To add more samples to the list, there are two options:

1. Right click the sample list, and select "Append Sample".
2. With the last sample highlighted, press the down arrow on the keyboard. Click 'Yes' to append a sample.

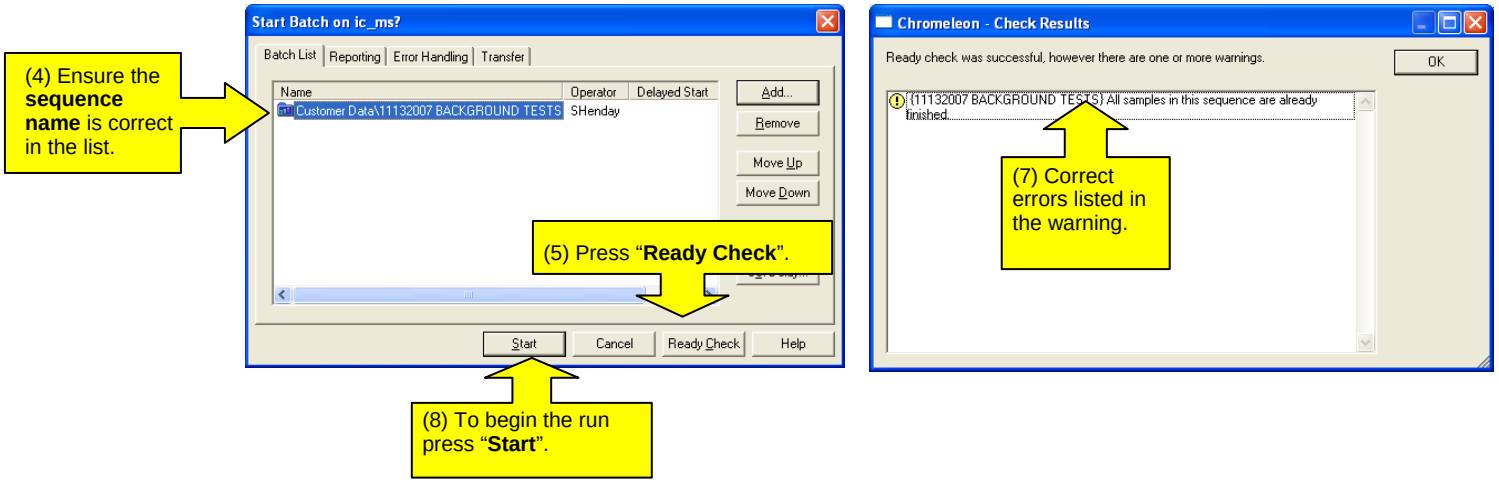
Starting a Sequence

From the Browser Screen

1. Locate the Batch Menu.
2. Click “Start…”


(1) Locate the **Batch** Menu
Alternatively, press this icon of a list of pages with a green arrow as a shortcut to “Batch” → “Start”.
Chromeleon - [Sequence1]
File Edit View Workspace Qualification Batch Tools Window Help
Edit... Ctrl+B
Start...
Starts the sample batch
(2) Select “**Start**” from the menu.

3. The Batch List screen opens.
4. The name of your sequence appears in the list.
5. Click the “Ready Check” button. Among many other checks, this ensures:
    - There are no errors in the program
    - All modules are connected to the software
    - All lamps are on for the UV.
    - You are not running a manual background acquisition.
6. If the Ready Check is successful, you will get a message of success.
7. If the Ready Check fails, you will receive a list of errors and warnings. If only warnings appear, clicking OK will start the sequence. To correct errors and warnings, click “Cancel” and address the errors and warnings. Perform another ready check.
8. Press Start.


(4) Ensure the **sequence name** is correct in the list.
Start Batch on ic_ms?
Batch List Reporting Error Handling Transfer
Name Operator Delayed Start
Customer Data\11132007 BACKGROUND TESTS SHenday
Add... Remove Move Up Move Down
(5) Press “**Ready Check**”.
Start Cancel Ready Check Help
(8) To begin the run press “**Start**”.
Chromeleon - Check Results
Ready check was successful, however there are one or more warnings.
OK
(11132007 BACKGROUND TESTS) All samples in this sequence are already finished.
(7) Correct errors listed in the warning.

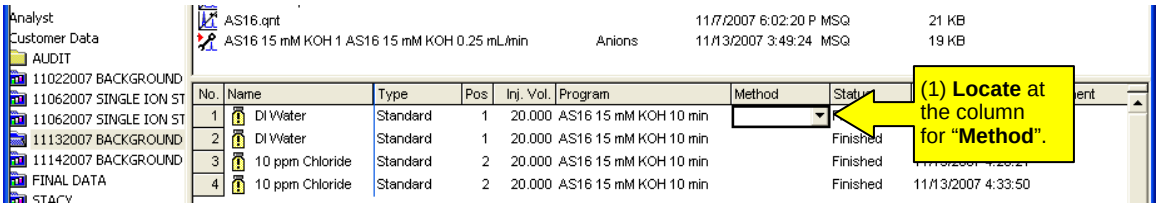
4.1 Examining Data Files

After the samples have been run, the status changes from “Single” to “Finished”. This section walks you through data analysis, and how to examine finished samples.

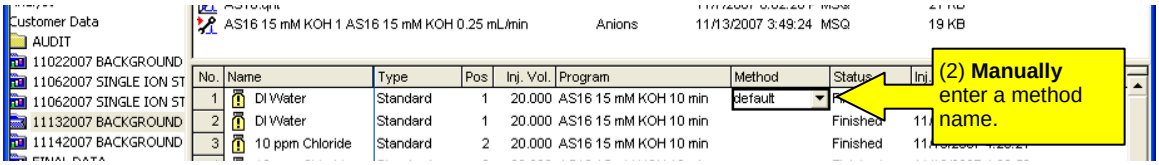
A quantification method must be specified in the sequence before the data file can be opened

To check if the sequence has a quantification file associated with the samples:

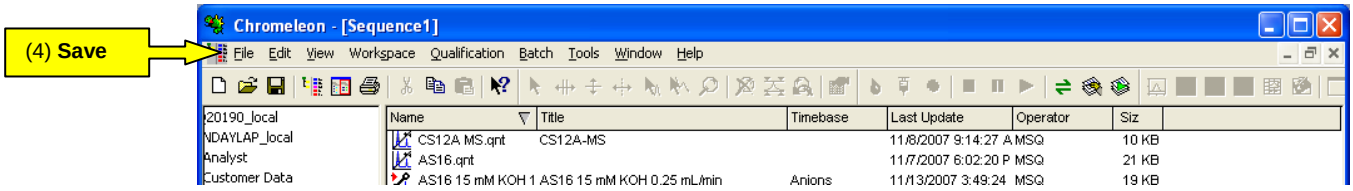
1. Open the sequence. If the column under “Method” is blank, then there is no method associated with the sample.



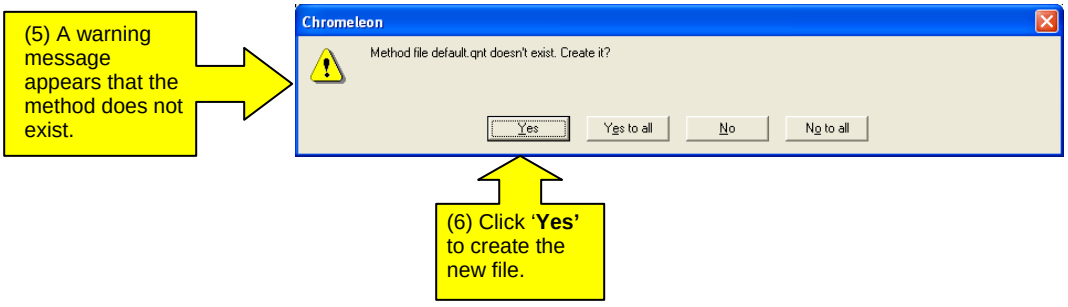
2. The fastest way to create a method is to type a name in the column, and hit enter.
3. Click F9 to add the same method name down the entire column.



4. Then File → Save the sequence.



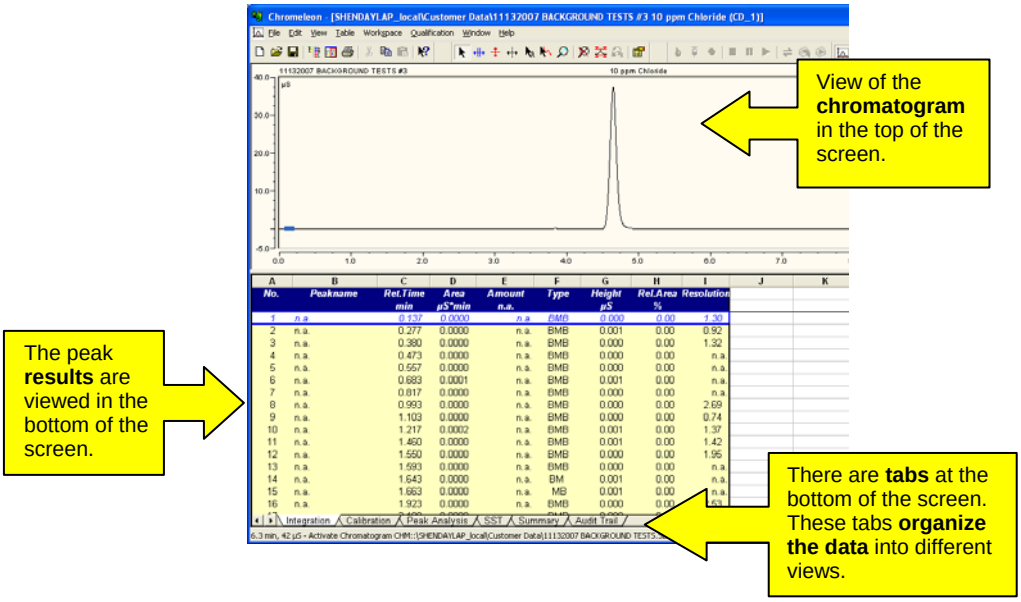
5. The software alerts you that there is no method file with the name you entered, and asks if you would like to create the file.
6. Click Yes.


(5) A warning message appears that the method does not exist.
Chromeleon
Method file default.qnt doesn't exist. Create it?
Yes
Yes to all
No
No to all
(6) Click ‘**Yes**’ to create the new file.

Examining Data Files (continued…)

With the method defined in the sequence, simply double click on the sample in the sequence to open the chromatogram.

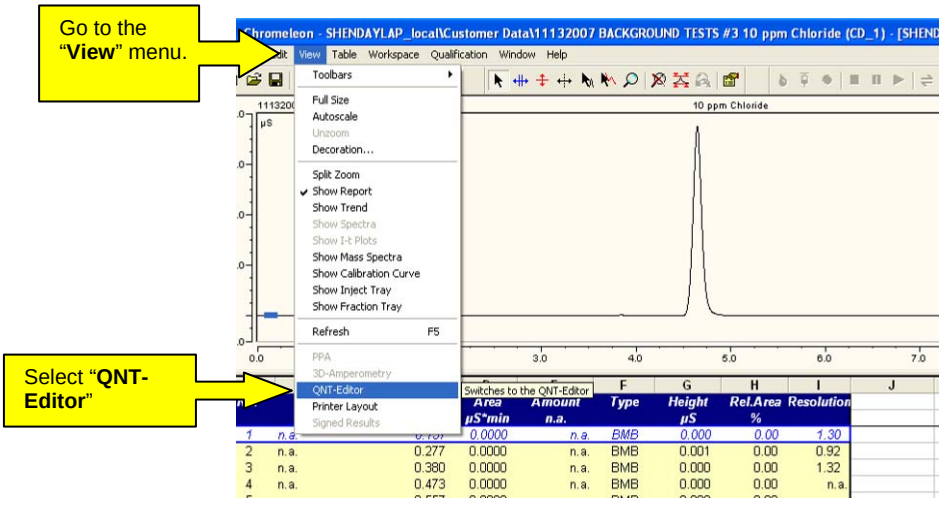
This view is known as the "Integration View". This screen is purely for viewing the data and the results.


View of the **chromatogram** in the top of the screen.
The peak **results** are viewed in the bottom of the screen.
There are **tabs** at the bottom of the screen. These tabs **organize the data** into different views.

In order to integrate the data, name the peaks, and define the points of the calibration curve, enter the quantification (QNT) view.

To enter the QNT view:

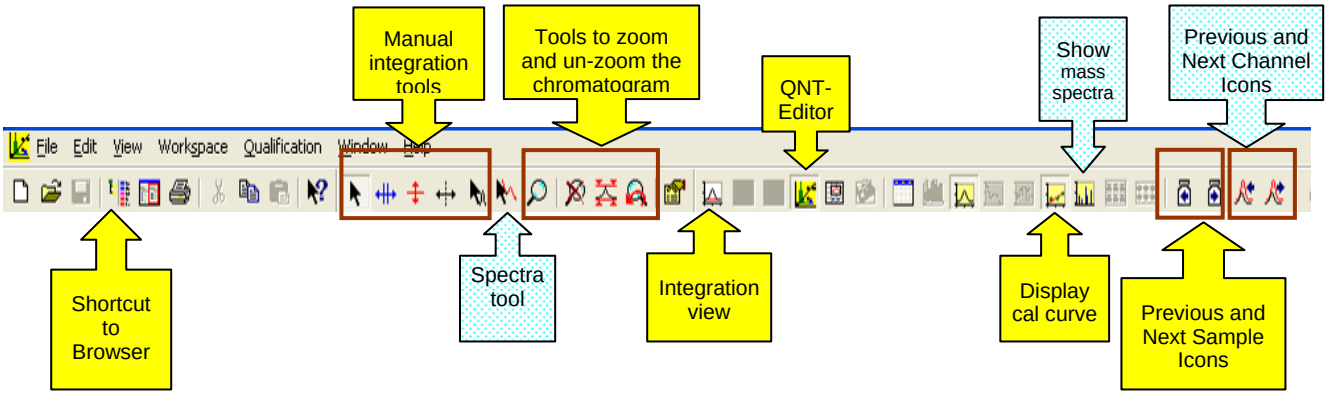
1. Locate View in the menu bar.
2. Select QNT-Editor.



As with the Integration view, the QNT-Editor view shows the chromatogram on the top of the screen and a series of tabs appears at the bottom of the screen. Unlike the Integration view, there are no data values in the tabs, rather the settings to define the peaks.

Before we summarize the function of each tab, let's examine the icon toolbar.

4.2 Toolbar



The labels of the icons that are used frequently with the mass spectrometer are shaded with dots.

Familiarize yourself with the various icons in the toolbar. If you are unsure about the function of an icon, hover the mouse over the icon and an informational dialog box names the icon (also referred to as the "tool tip").

The remainder of this section and the following on the QNT-Editor emphasize the use of these shortcut icons, so it will be useful to have knowledge of them before you continue.

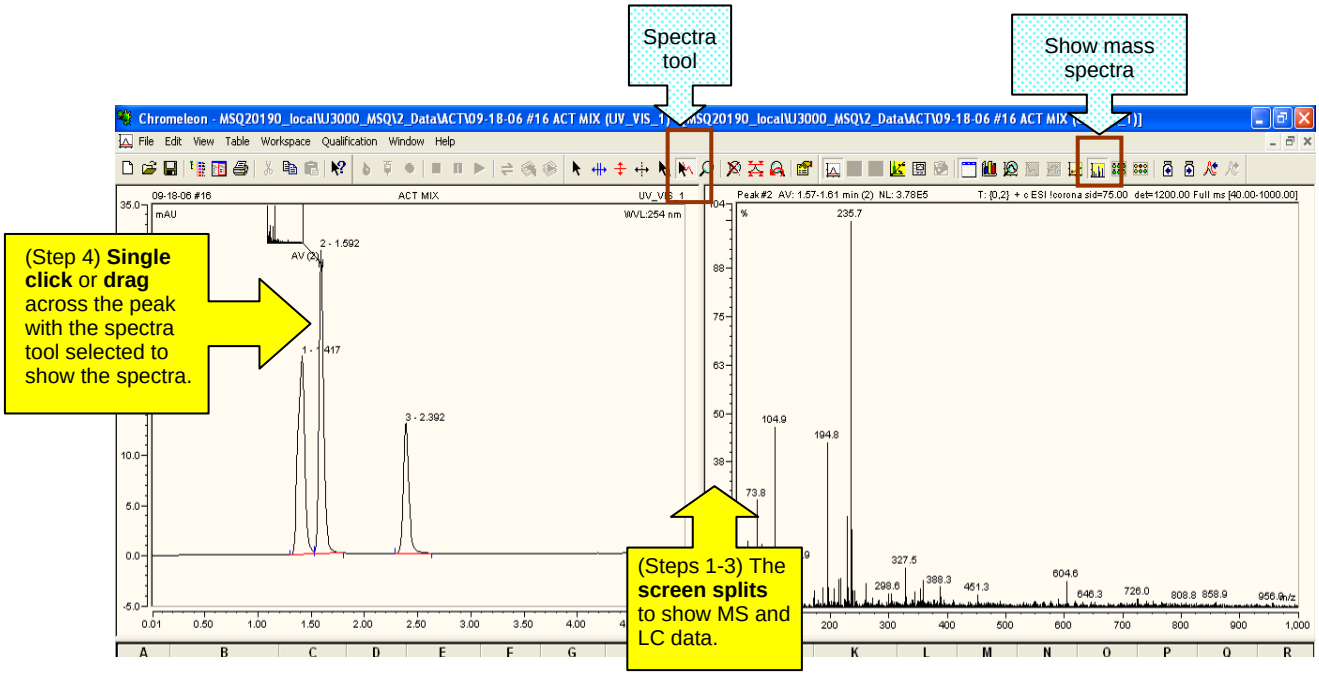
4.3 Mass Spectra Data Extraction [Integration and QNT-Editor Views]

In many cases, such as identification of unknown peaks, it is necessary to extract mass spectra data for a LC peak. For this to occur, full scan data ***must*** be acquired. (Hopefully the *m/z* of this known or unknown peak falls within the range of the full scan.)

THIS IS TYPICALLY THE FIRST STEP WHEN IDENTIFYING THE *m/z* OF A KNOWN OR UNKNOWN SAMPLE.

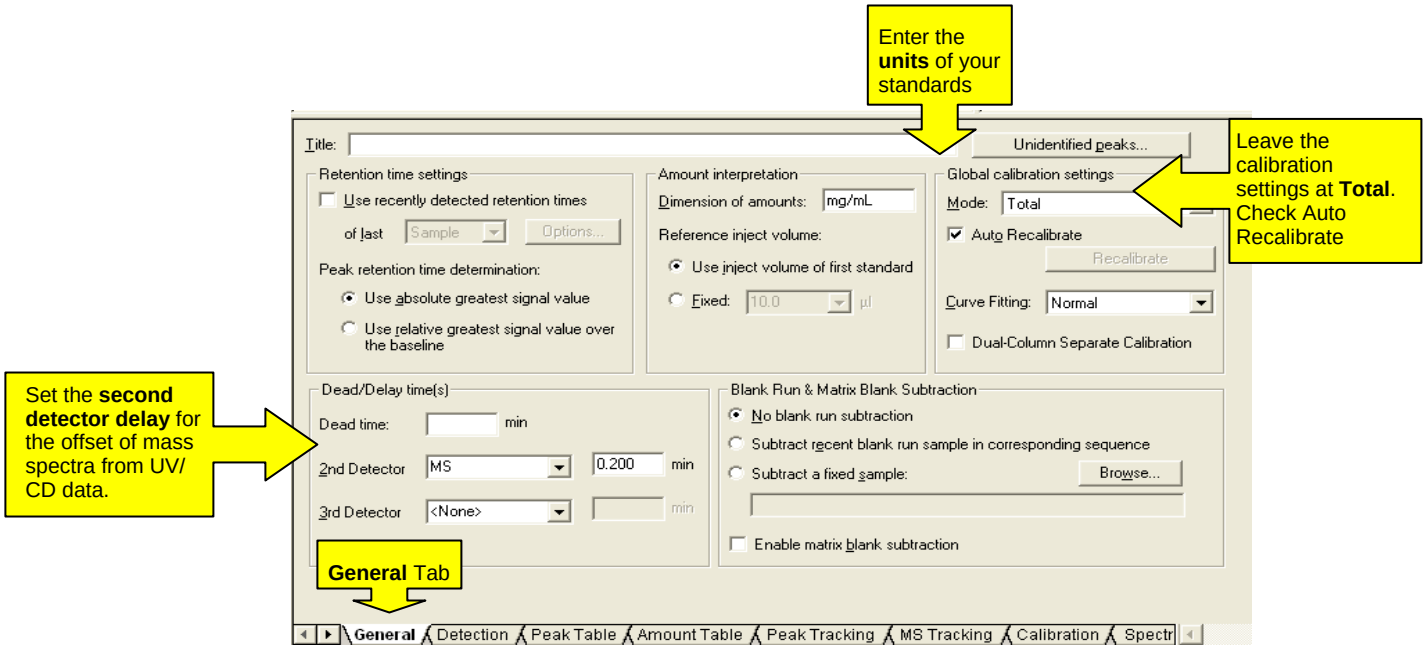
To extract mass spectrometer data:

1. Open the data file by double clicking on the finished sample in the Browser.
2. Go to the View Menu → Show Mass Spectra or click the "Show Mass Spectra" icon on the toolbar. The screen will change to show the CD/UV data on the left side of the screen and the mass spectra data on the right side of the screen.
3. Select the spectra tool from the toolbar.
4. Single left click the apex of the UV/CD peak or, to average the spectra across the peak, hold the left mouse key down and drag across the width of the peak.


Spectra tool
Show mass spectra
(Step 4) **Single click** or **drag** across the peak with the spectra tool selected to show the spectra.
(Steps 1-3) The **screen splits** to show MS and LC data.

5. The mass spectra data on the right side of the screen updates, showing the spectra for the peak generated by the left mouse click of step 4.
6. Identify the most intense peak of the spectra.
7. Single left click on the most intense ion of the mass spectra to extract that single ion as a chromatogram (ion intensity vs. time) out on the left side of the screen.
8. If the scale is offset too much by overlaying mass spectra data with CD/UV data:
    a. **Right click on the UV/CD data** overlay with mass spectrometer data.
    b. Select "**Decoration**…" from the context menu.
    c. Locate the "**Comparison**" tab.
    d. On the Comparison tab, find the box for "**Overlay**". Select "**Overlay with right signal axis**" and click OK.
    e. UV/CD data is displayed on the left scale, MSQ data on the right scale.

General Tab



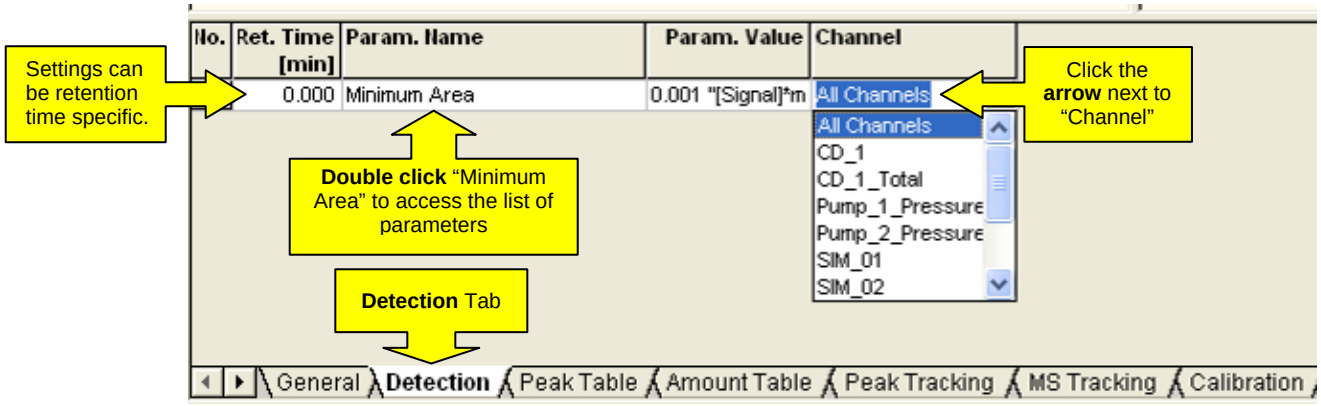
Dimension of amounts: Units for the analysis (e.g., ppm, µg/mL)

Global calibration settings: Defines which points are to be used to generate the calibration curve. Leave as the default "Total" with "Auto Recalibrate" checked.

"2nd Detector" Delay: It is important to match the retention times of the UV/CD data and the mass spectrometer. (The tubing and flow rate affect this value. If they are changed, reoptimize this delay time.)

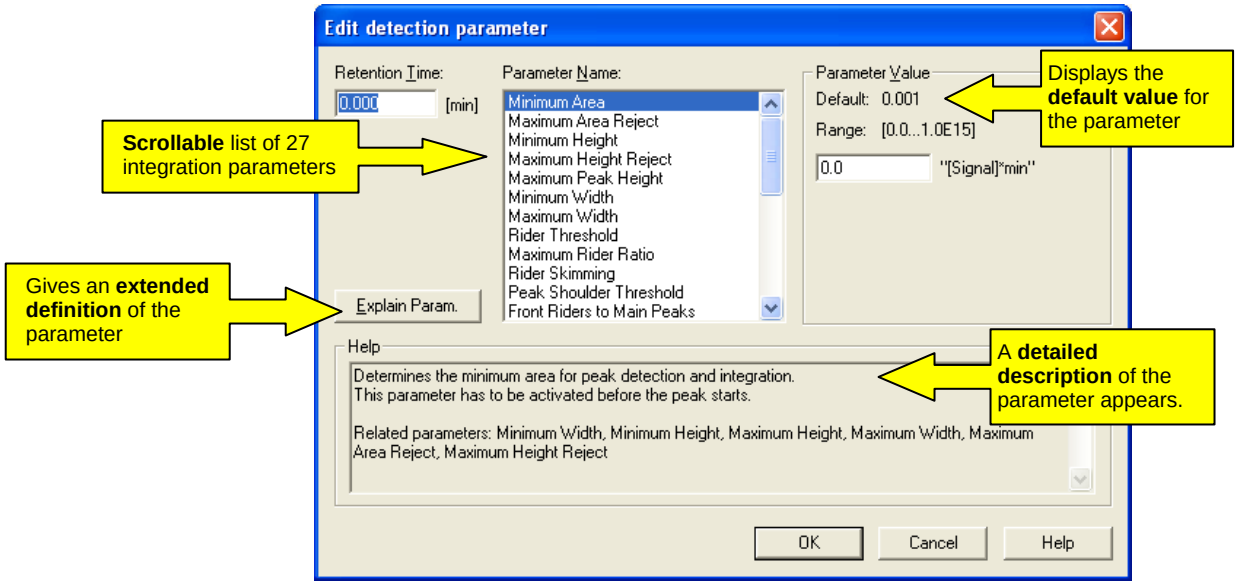
1. Overlay the UV/CD peak with the MS peak.
    a. With the UV/CD data open, *press and hold* the "Ctrl" key on the keyboard while using the mouse to click the "Previous" or "Next" Channel icon. This overlays the multiple data channels of the same run.
    b. The data channels are organized alphabetically.
2. Manually calculate the difference in retention time between the LC and MS peaks.
3. Define "2nd Detector" as "MS" using the down arrow menu.
4. Click OK to the warning message that appears.
5. Enter the delay of time of MS data into the field.
6. Ensure the maxima of both peaks are aligned; adjust the value as required.

Detection Tab



The "Detection" tab defines the criteria for identifying peaks. There are over twenty five parameters available to define a peak in the chromatogram. These parameters are unique for each application, and must be user-customized for the analysis.

NOTE: To see a complete list of the parameters, along with explanation of the parameter, double click the parameter name field. *(Not the title, but the name of the parameter.)*



Use the retention time column to specify the time of the integration step. The detection parameters are time dependent. For example, Minimum Area can be set to different values throughout the integration of the chromatogram by having multiple retention times for the Parameter Name.

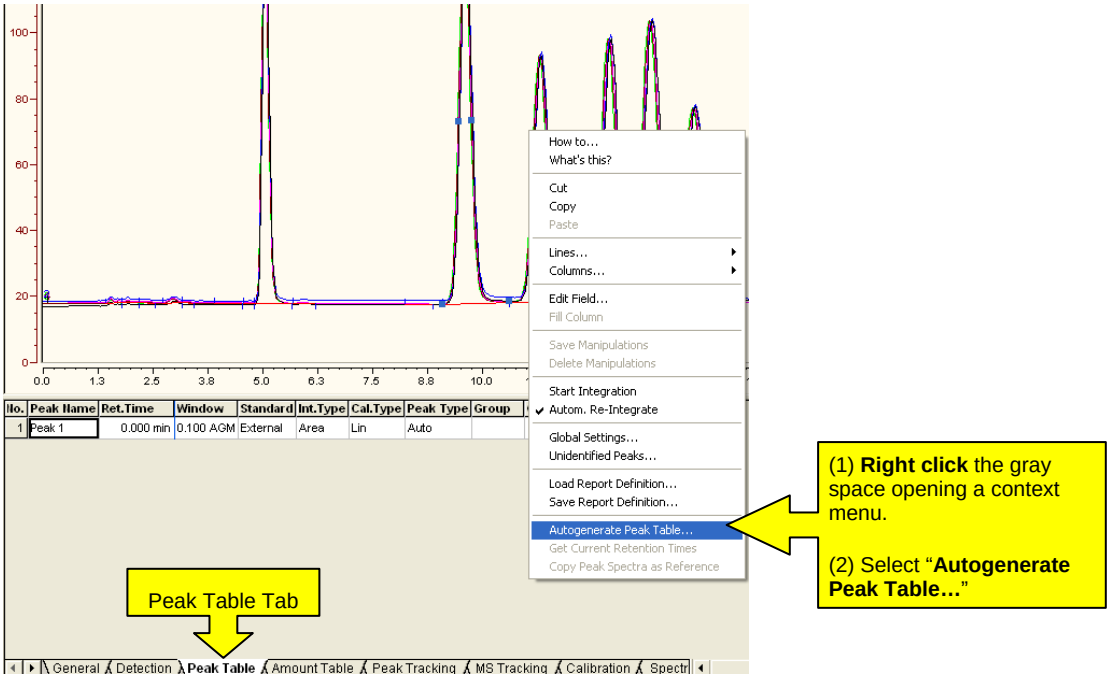
- ***Use the "Channel" column to specify the channel for the parameter***. Parameters can be custom tailored for each detection channel, so the integration for the UV data will not interfere with the integration of the MS data.

Peak Table Tab

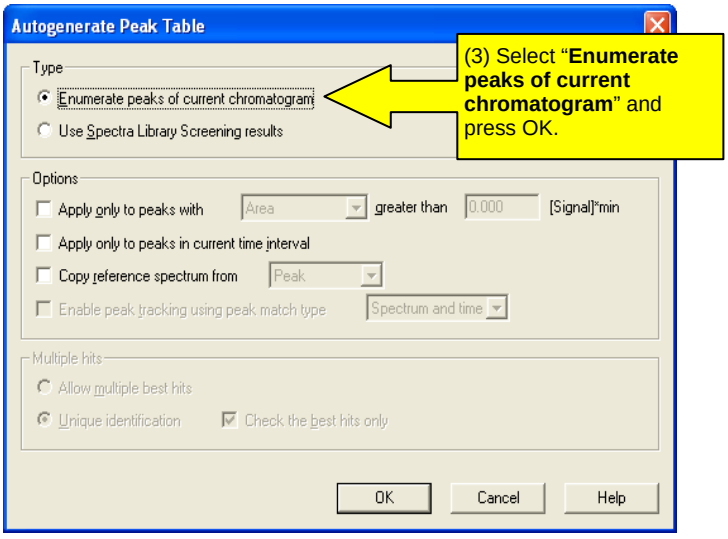
The "Peak Table" tab defines the criteria for labeling peaks based on retention time and how to draw the calibration curve fit.

In order to create a list of peaks with the retention time from the chromatogram:

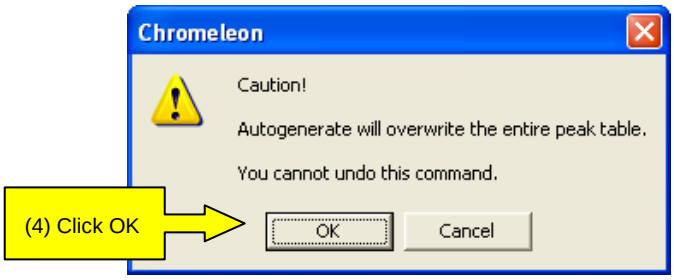
1. Right click on the grey space of the peak table to open the context menu.
2. Select "Autogenerate Peak Table…"



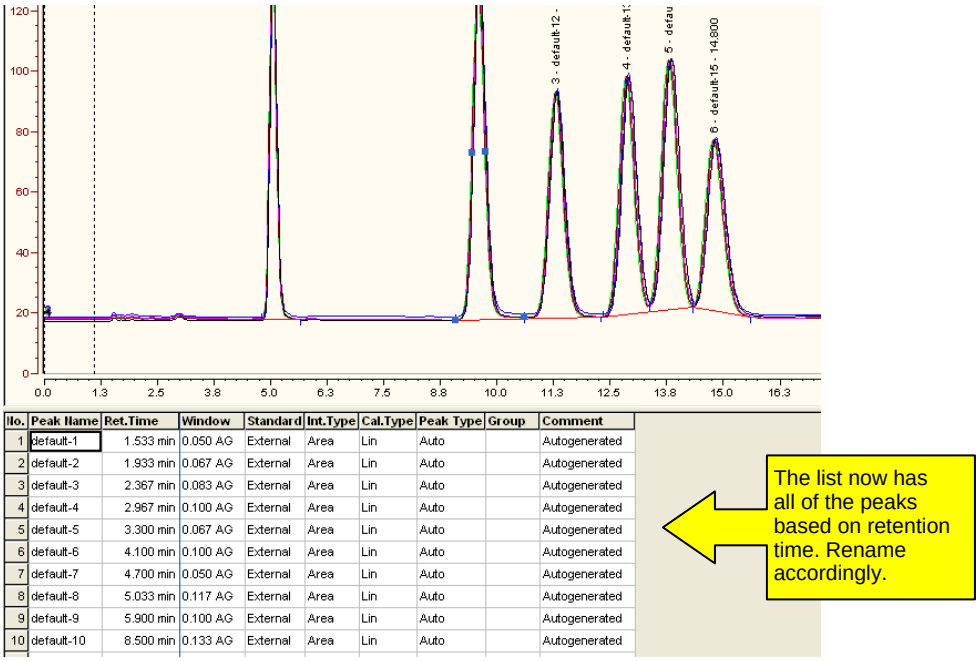
3. In the dialog box that appears, select the default of "Enumerate peaks of current chromatogram". Press OK.



4. Click OK to the warning message.



5. The peak table will generate rows based on how many peaks are defined in the chromatogram.
6. Rename the peaks from “default-1, 2, 3, etc” to the name of the analyte.
7. Adjust the peak window if required.


3 - default-12 -
4 - default-1
5 - defau
6 - default-15 - 14.800
The list now has all of the peaks based on retention time. Rename accordingly.

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Peak Type	Group	Comment
1	default-1	1.533 min	0.050 AG	External	Area	Lin	Auto		Autogenerated
2	default-2	1.933 min	0.067 AG	External	Area	Lin	Auto		Autogenerated
3	default-3	2.367 min	0.083 AG	External	Area	Lin	Auto		Autogenerated
4	default-4	2.967 min	0.100 AG	External	Area	Lin	Auto		Autogenerated
5	default-5	3.300 min	0.067 AG	External	Area	Lin	Auto		Autogenerated
6	default-6	4.100 min	0.100 AG	External	Area	Lin	Auto		Autogenerated
7	default-7	4.700 min	0.050 AG	External	Area	Lin	Auto		Autogenerated
8	default-8	5.033 min	0.117 AG	External	Area	Lin	Auto		Autogenerated
9	default-9	5.900 min	0.100 AG	External	Area	Lin	Auto		Autogenerated
10	default-10	8.500 min	0.133 AG	External	Area	Lin	Auto		Autogenerated

a. “Window” is the span of time around the total retention time that the software will examine to label your peak. (*e.g.*, If the Ret. Time is set to 4.00 minutes, with a Window of 0.500 min, the software will look at all data between 3.75-4.25 minutes to label your peak.)

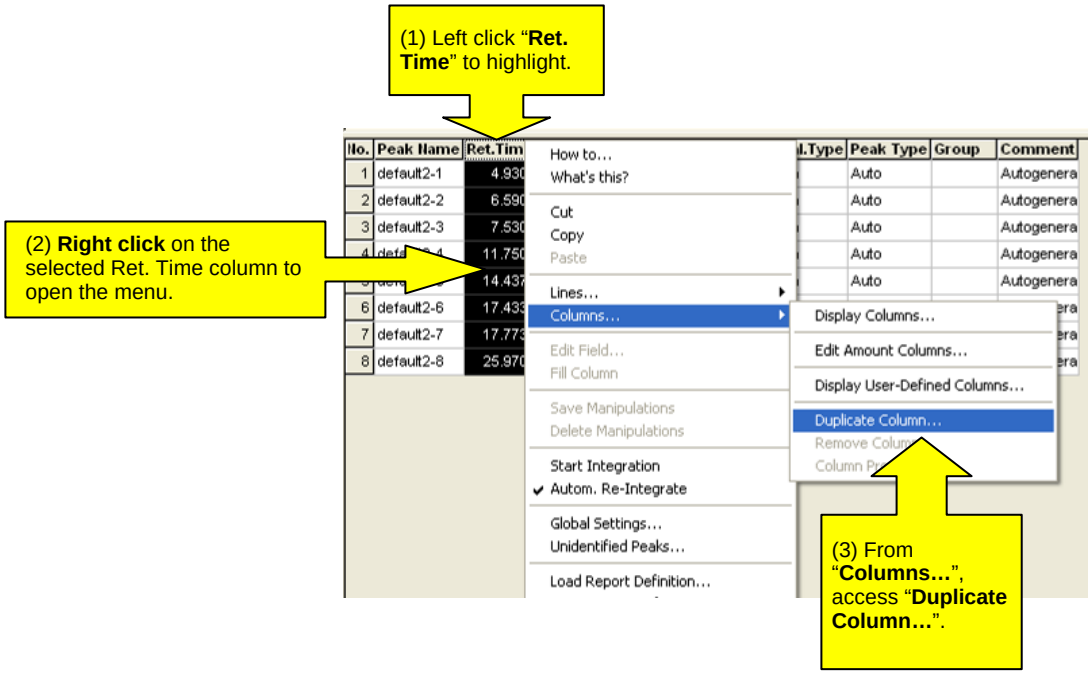
b. Additional parameters for Window can be found by double clicking the listed window.

Defining Internal Standards in the Peak Table Tab

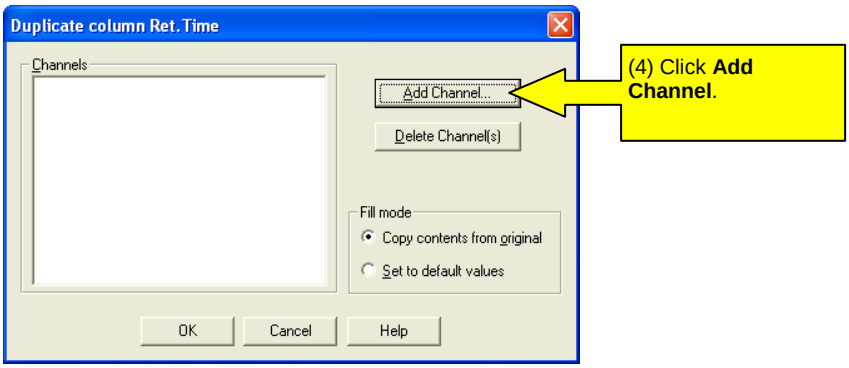
Using internal standards is crucial when quantifying peaks by mass spectrometry. An internal standard is a substance that is added in known (identical) quantities to your standard and unknown samples. The addition of internal standards corrects for sample variation due to matrix effects, fouled entrance source, etc. In mass spectrometry, the ideal internal standard is an isotopically labeled substance, so it will have a similar retention time and chemical behavior as the analyte of interest.

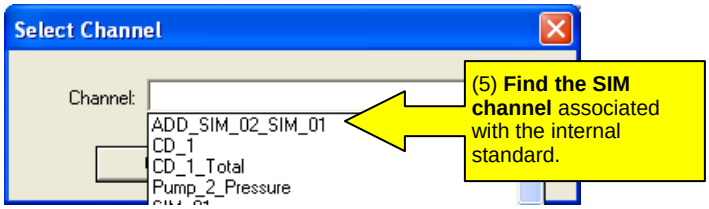
Internal standards are set up as a SIM scan in the mass spectrometer. In order to quantify the data using the internal standard, the following steps must be taken:

1. Left-click “Ret. Time” to select that column.
2. Right-click the “Ret. Time” column to open the context menu.
3. Go to “Columns… and select “Duplicate Column”.
4. Click “Add Channel”. Then use the pull down menu to select the SIM channel of the internal standard. Click OK.
5. Click OK to the “Duplicate column retention time” box.
6. At this point, the column for retention time has been duplicated for that specific SIM channel. Delete all values from the column except the retention time of the analyte in that SIM channel.

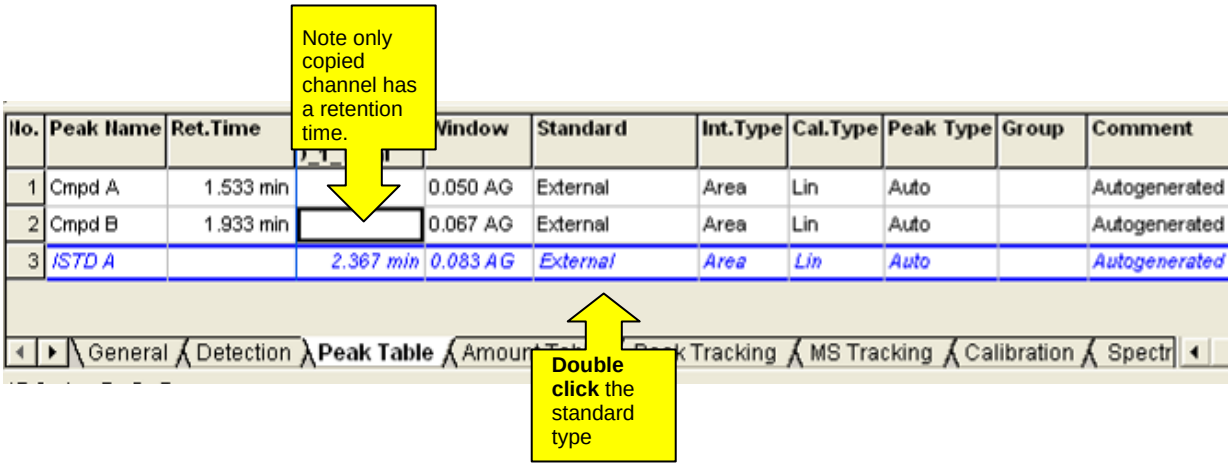


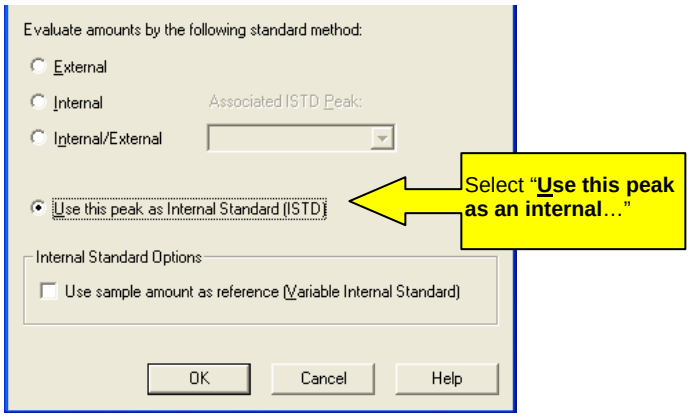
   a. Delete the retention time of the internal standard from the original retention time column.
   b. Only the SIM channel for the internal standard should have a retention time entered.



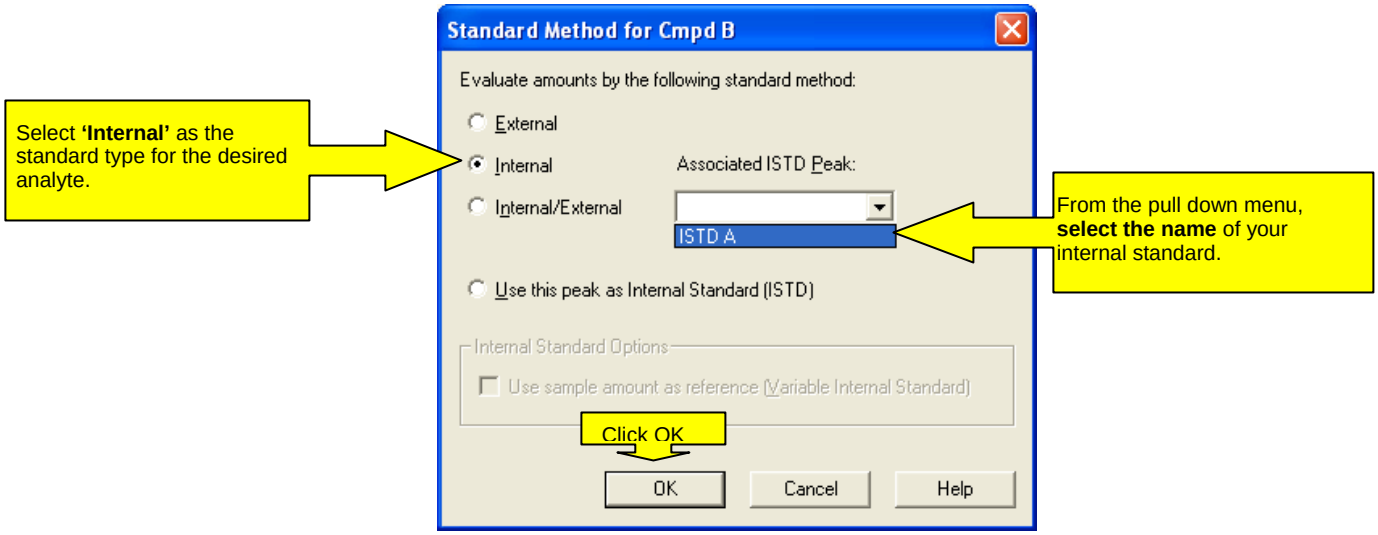


7. In complicated analysis, compound identification may be easier if separate SIM channels are created for each analyte of interest.
8. Locate the "Standard" column.
    a. Chromeleon defaults to list the "Standard" as 'External'.
    b. Double click on the word "External" in internal standard row.
    c. Select "Use this peak as Internal Standard (ISTD)". Click OK.
    d. The color of the row shades in yellow when internal is selected.

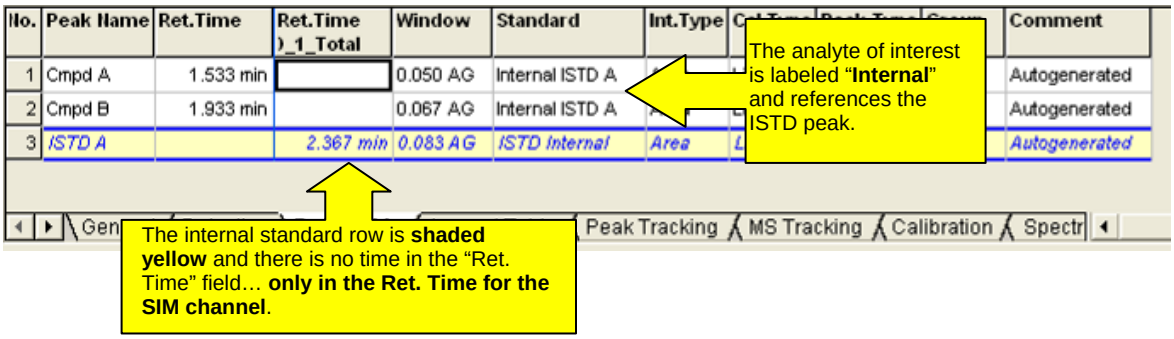

Note only copied channel has a retention time.
No. | Peak Name | Ret.Time | Window | Standard | Int.Type | Cal.Type | Peak Type | Group | Comment
1 | Cmpd A | 1.533 min | 0.050 AG | External | Area | Lin | Auto | | Autogenerated
2 | Cmpd B | 1.933 min | 0.067 AG | External | Area | Lin | Auto | | Autogenerated
3 | ISTD A | 2.367 min | 0.083 AG | External | Area | Lin | Auto | | Autogenerated
General
Detection
Peak Table
MS Tracking
Calibration
Double click the standard type



9. The last step is to link the analytes of interest to the internal standard.
   a. Double click on the standard column for the analyte of interest.
   b. In the dialog box, select "Internal" and in the pull down list for "Associated ISTD Peak" choose the name of the internal standard.


Standard Method for Cmpd B
Evaluate amounts by the following standard method:
External
Internal
Associated ISTD Peak:
Internal/External
ISTD A
Use this peak as Internal Standard (ISTD)
Internal Standard Options
Use sample amount as reference (Variable Internal Standard)
OK
Cancel
Help
Select 'Internal' as the standard type for the desired analyte.
From the pull down menu, select the name of your internal standard.
Click OK

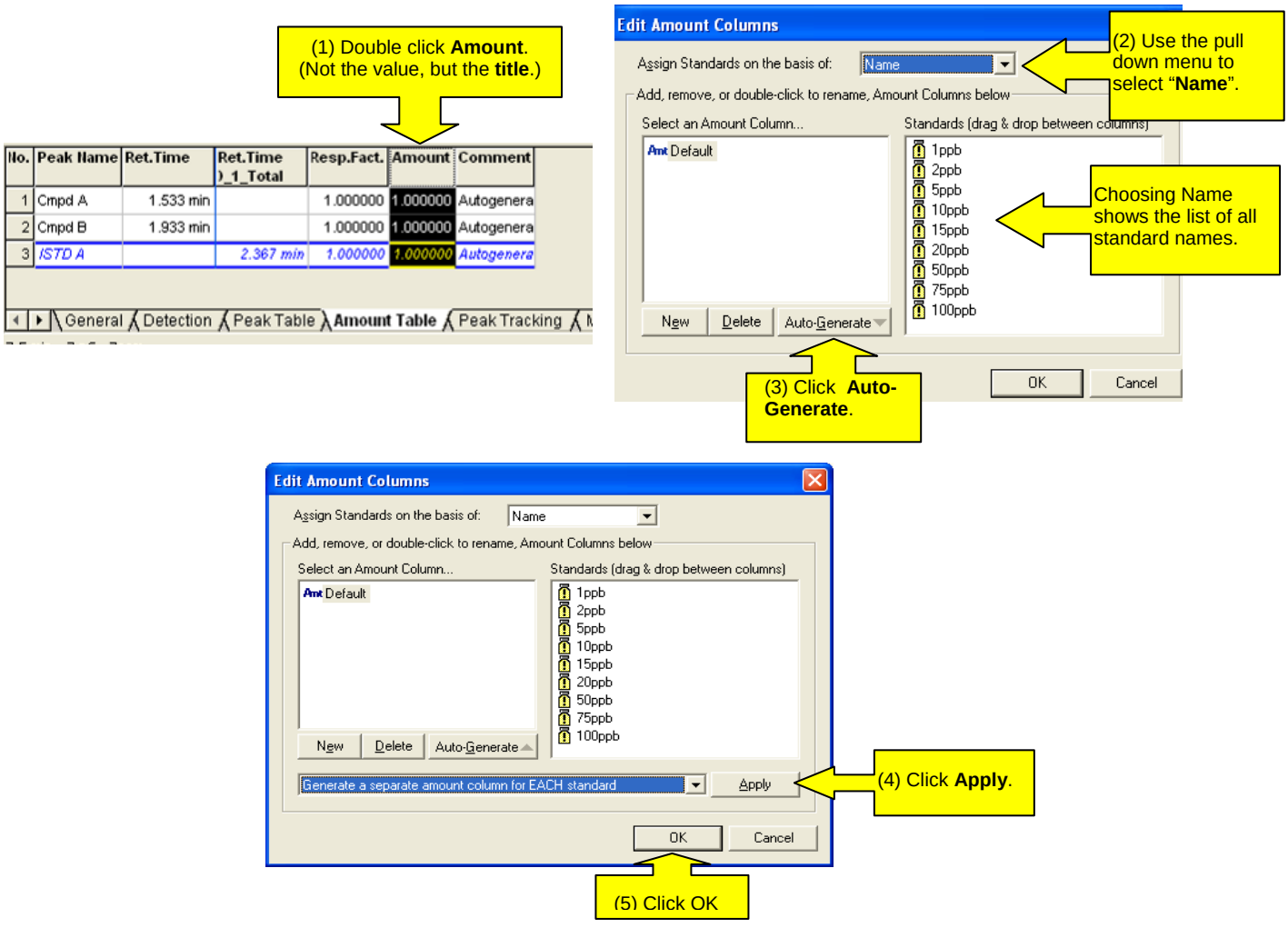
10. When complete: standard type for the UV/Conductivity as "External", the SIM monitored analyte as "Internal" referencing the internal standard peak and the internal standard labeled as "ISTD Internal".


No.
Peak Name
Ret.Time
Ret.Time)_1_Total
Window
Standard
Int.Type
Comment
1 Cmpd A 1.533 min 0.050 AG Internal ISTD A Autogenerated
2 Cmpd B 1.933 min 0.067 AG Internal ISTD A Autogenerated
3 ISTD A 2.367 min 0.083 AG ISTD Internal Area Autogenerated
Peak Tracking
MS Tracking
Calibration
Spectr
The analyte of interest is labeled "Internal" and references the ISTD peak.
The internal standard row is shaded yellow and there is no time in the "Ret. Time" field… only in the Ret. Time for the SIM channel.

Amount Table Tab

The amount table is used to define the concentration of the standards.

1. Double click on the column labeled “Amount”. As long as standards have been defined in the sequence, the “Edit Amount Columns” menu appears.
2. Use the pull down menu to assign standards on the basis of “Name”. Once they are assigned on the basis of name, the list of the standards appears on the right side of the dialog box.
3. Click Auto-Generate to open another option on the screen.
4. Click Apply to “Generate a separate amount column for EACH standard.”
5. Click OK.



6. Columns with the standard names appear on the Amount Table Tab. Enter the amount information into the appropriate columns and rows. If using an internal standard, be sure to specify the same concentration for the internal standard for each concentration.

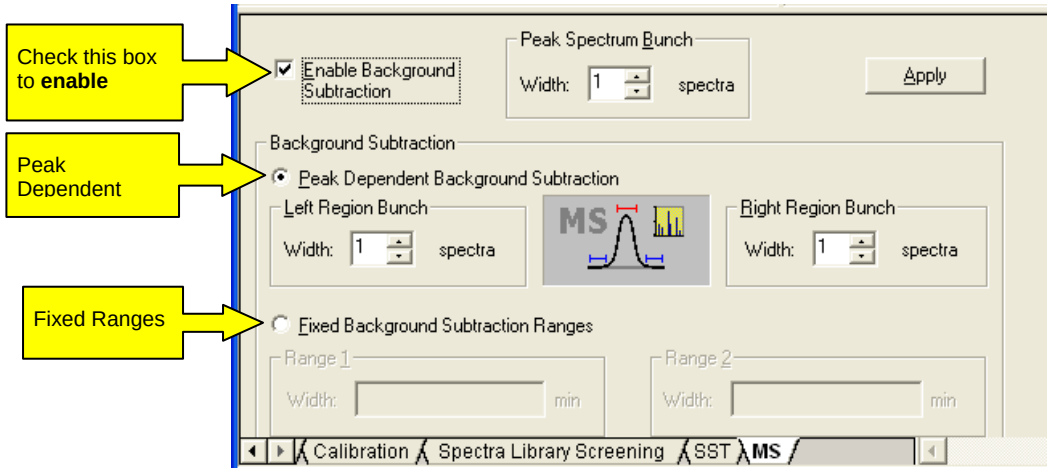
Background Subtraction

In some circumstances, it may be useful to subtract the background ions out of the spectra. Subtracting the background ions may make it easier to identify what masses are attributed to your sample, versus those attributed to the solvents.

There are two ways to subtract the background spectra:

1. via the MS tab in the QNT editor
2. via Mouse Clicks on the chromatogram

To Enable Background Subtraction via MS Tab



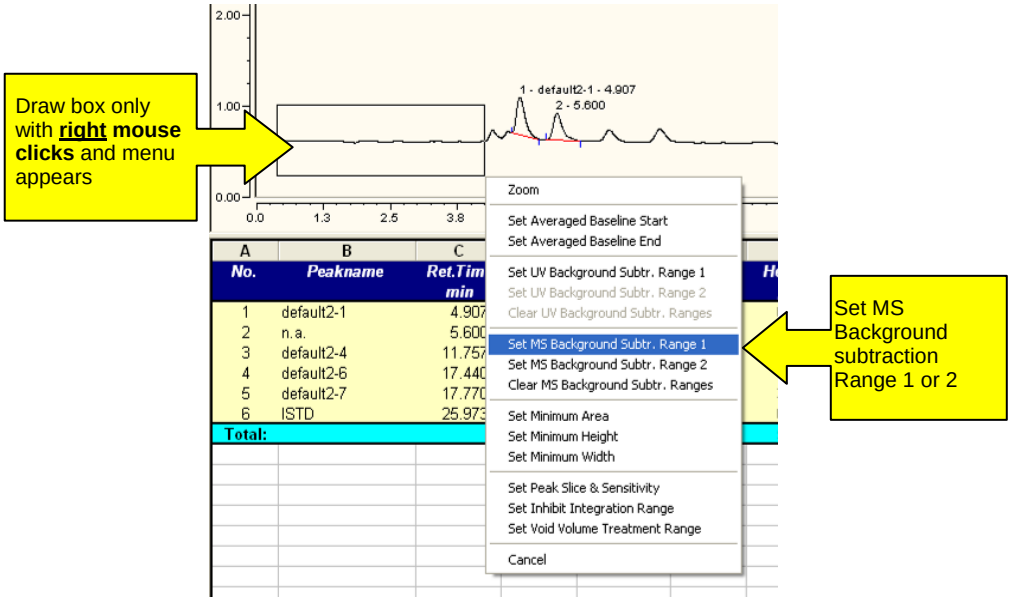
The MS tab defaults to background subtraction disabled. To activate background subtraction:

1. Click the box next to “Enable Background Subtraction”.
2. Two options appear for background subtraction:
    a. **Peak Dependent**: The software will average a user defined amount of spectra points to the left and right of each peak. Automatically calculated for each peak in the chromatogram.
    b. **Fixed Background**: Subtracts two specific time segments from the chromatogram. (*Typically the time points are set at the beginning and end of an analytical run).*

To Enable Background Subtraction via Mouse Clicks

Please note: Only fixed background can be enabled via mouse clicks.

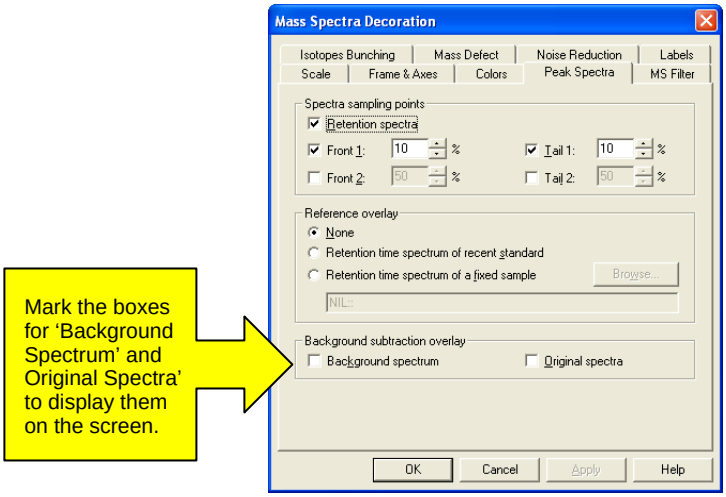
1. View the chromatogram in either Integration or QNT-Editor mode.
2. Identify an area of the baseline to subtract.
3. **Press and hold** the right mouse button at the beginning of this area. Without letting go of the mouse, draw a box around the baseline region.
4. Release the right button of the mouse, but do not click anywhere.
5. A context menu appears next to the box. Select "Set MS Background Subtraction Range 1".
6. If a second range is desired, repeat steps 2–4. In the menu select "Set MS Background Subtraction Range 2".

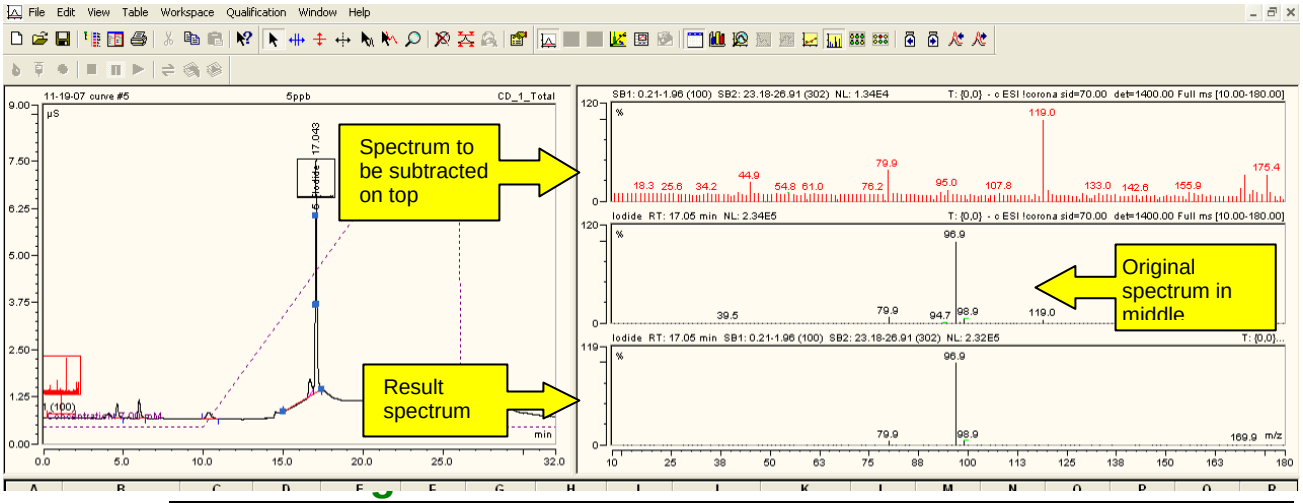

Draw box only with right mouse clicks and menu appears
1 - default2-1 - 4.907
2 - 5.600
Zoom
Set Averaged Baseline Start
Set Averaged Baseline End
Set UV Background Subtr. Range 1
Set UV Background Subtr. Range 2
Clear UV Background Subtr. Ranges
Set MS Background Subtr. Range 1
Set MS Background Subtr. Range 2
Clear MS Background Subtr. Ranges
Set Minimum Area
Set Minimum Height
Set Minimum Width
Set Peak Slice & Sensitivity
Set Inhibit Integration Range
Set Void Volume Treatment Range
Cancel
Set MS Background subtraction Range 1 or 2

To View the Subtracted Spectra

1. Go to View. Select "Show mass spectra" *or* press the show mass spectra icon.
2. Right click on the mass spectra to open the context menu.
3. Select "Decoration" from the menu.
4. Locate the "Peak Spectra" tab.

5. Check the boxes for “Background spectrum” and “Original spectra”.

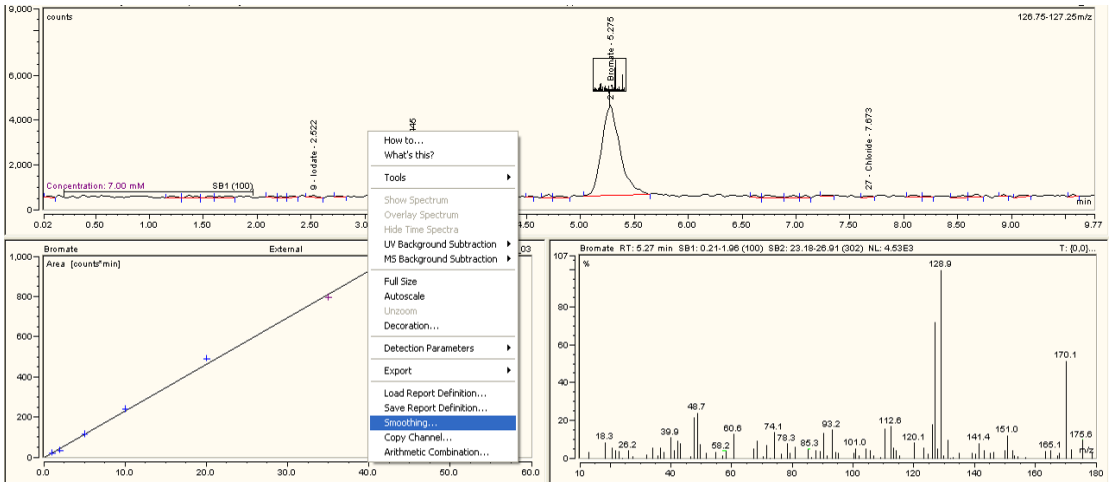


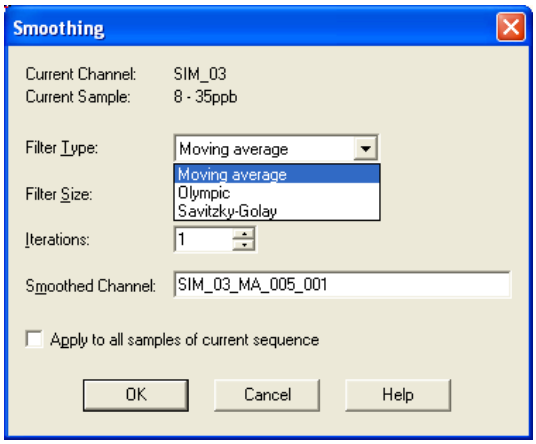

File Edit View Table Workspace Qualification Window Help
Spectrum to be subtracted on top
Result spectrum
Original spectrum in middle

As previously noted, the raw data can be smoothed as the data is collected. If you prefer, the data can be smoothed post data acquisition. Smoothing data can help produce a uniform peak shape.

1. Open a data file to the SIM channel (or desired data channel to smooth).

2. Right click on the chromatogram to open a context menu, select “Smoothing…”.
3. A menu will appear. Select the Filter Type from the pull down menu. Hit F1 for





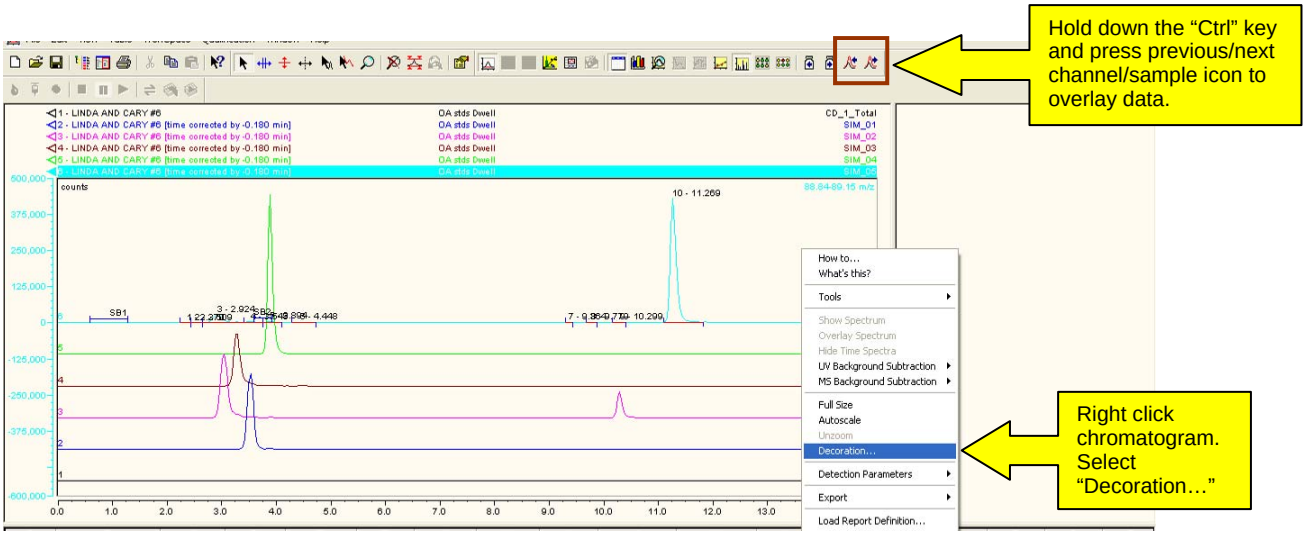
definitions of the filters.

4. **Filter Size**: The amount of points to smooth; keep this number as low as possible.
5. **Iterations**: How many cycles to smooth the data (e.g. selecting 2 will smooth the smoothed data).
6. **Smoothed channel**: The name of the new channel. If you do not want to create a new channel, then name it the same as the data channel you are smoothing, but this will overwrite your data!

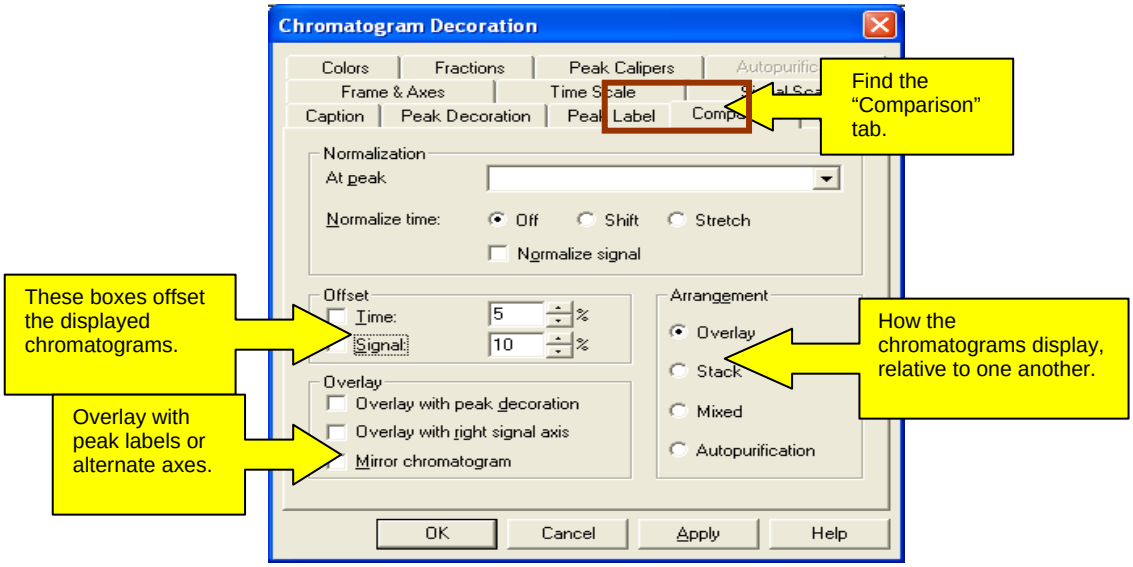
Overlaying Data Channels or Multiple Injections

To visually compare data from finished samples, follow these steps:

1. Open a finished sample file.
2. While holding down the “Ctrl” key on the keyboard, click the icon for the previous/next channel or previous/next sample. For ease in sorting, the channels are arranged alphabetically.
3. The data defaults to overlay with a signal offset of 10% per chromatogram. To adjust the display, right click on the chromatogram and open the context menu.
4. Select “Decoration” from the list.



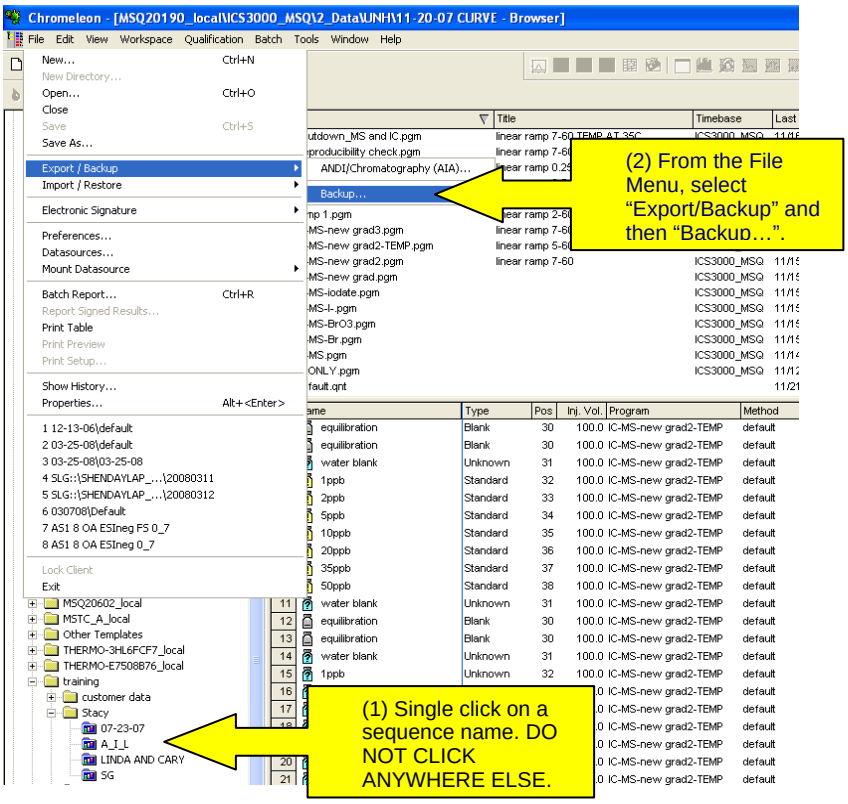
5. Locate the “Comparison” tab. Uncheck “Signal Offset”, if you would not like to see the baseline of the runs shifted up, but at the same level. Play with all of these parameters to see which one best fits your needs. (Including the arrangement section of the menu.)


Chromatogram Decoration
Colors
Fractions
Peak Calipers
Frame & Axes
Time Scale
Caption
Peak Decoration
Label
Find the "Comparison" tab.
Normalization
At peak
Normalize time:
Off
Shift
Stretch
Normalize signal
Offset
Time:
5
%
Signal:
10
%
These boxes offset the displayed chromatograms.
Arrangement
Overlay
Stack
Mixed
Autopurification
How the chromatograms display, relative to one another.
Overlay
Overlay with peak decoration
Overlay with right signal axis
Mirror chromatogram
Overlay with peak labels or alternate axes.
OK
Cancel
Apply
Help

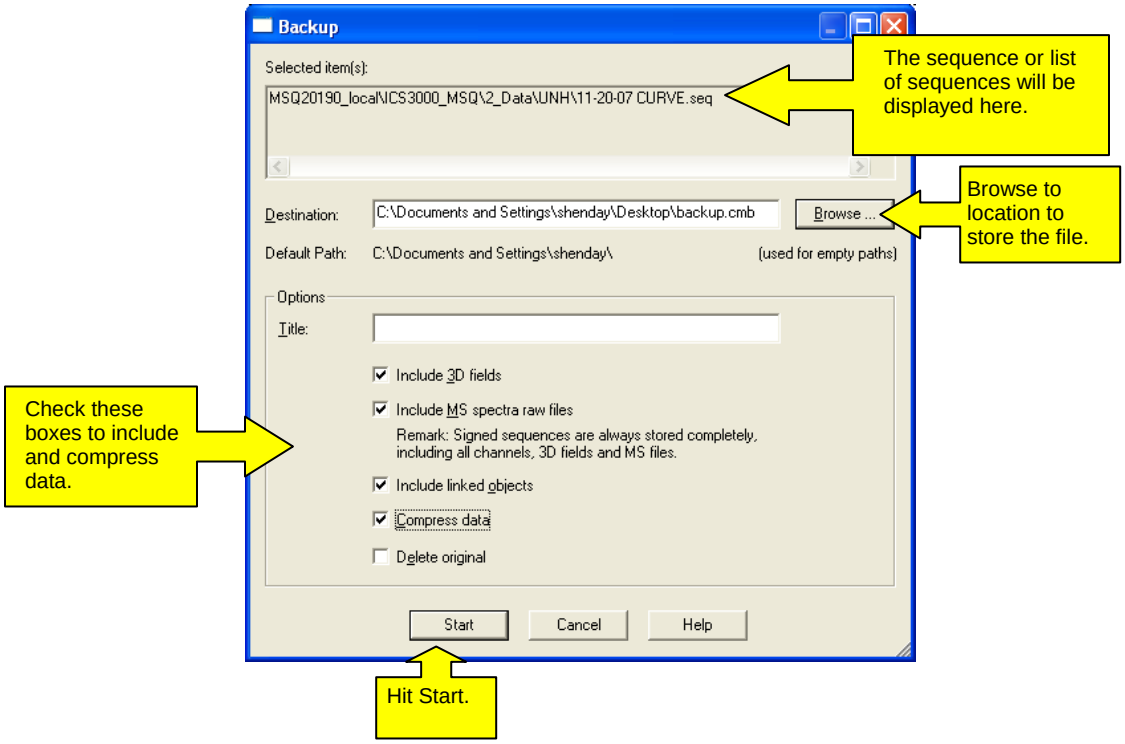
Backing Up/Exporting Data Files

In order to store the complete data, or e-mail the data to a Dionex representative, the following steps must be followed.

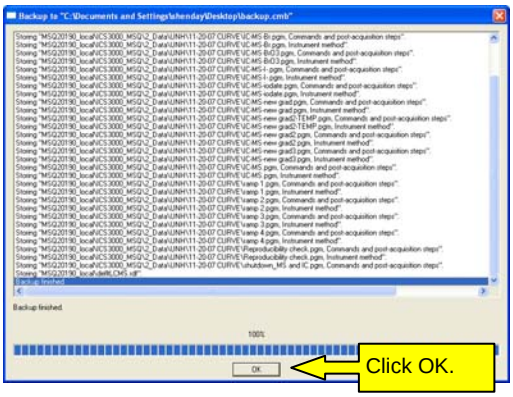
1. In the Browser screen, single click on the name of the sequence. Do not click anywhere else. If, for instance, you click on the name of a sample, then only that specific sample will be stored.
2. Go up to File and select “Export/Backup”
3. Choose “Backup…”



4. Selecting Backup opens a menu. Hit the Browse button to identify where the backup file should be stored and to name the file.
5. Make sure to check the boxes for:
    a. “Include MS spectra raw files”
    b. “Include linked objects”
    c. “Compress Data”



6. If by accident "Delete original" is checked, do not worry. Chromeleon will perform a safety check function to make sure that all backed up data is complete and correct before deleting your data.
7. Press Start.
8. As the data is backed up, a menu will show the status. Click OK when complete.

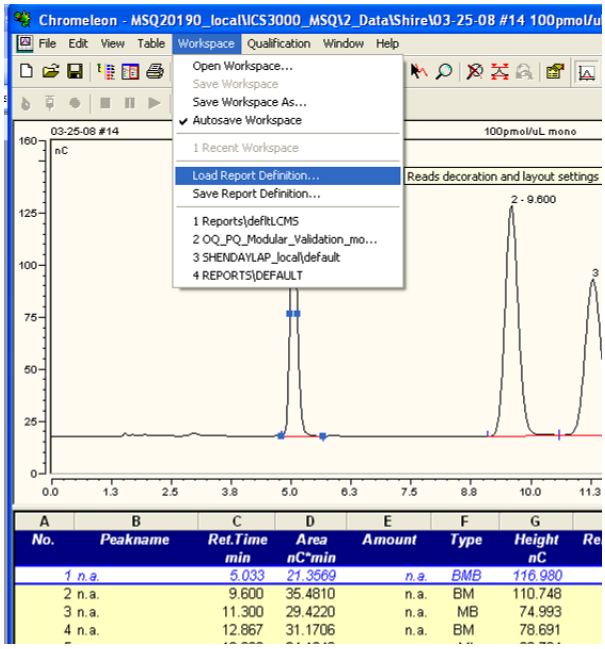


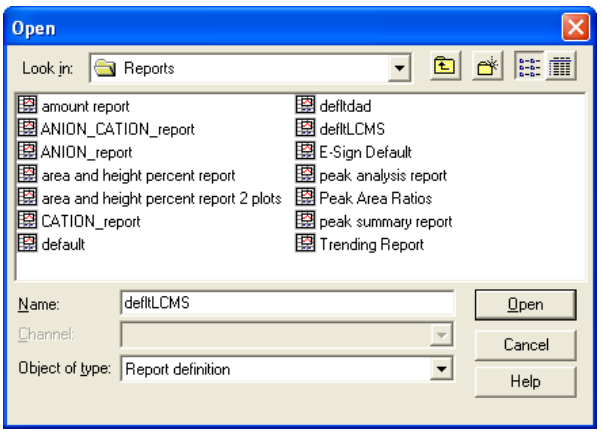
9. The data is stored in Chromeleon format, where it can only be opened on computers with Chromeleon software loaded. To import, simply double click the icon and Chromeleon will auto-launch, asking where to restore the file.

Report Definitions

There are limitless options for arranging the data in the printer layout screen. It may be easiest to start with the Dionex template.

1. Open a data file.
2. From the “Workspace” menu, select “Load Report Definition”.
3. Browse the directories until you reach the locked Dionex Templates folder. Open this folder and then select “Reports”. Open “defltLCMS”.
4. Click “No” to the option to save printer layout changes.

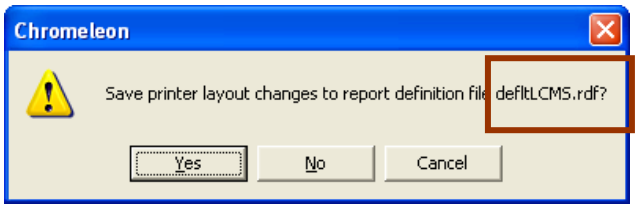




5. Go to the View menu and select “Printer Layout”.
6. Scroll through the tabs at the bottom of the screen to discover all of the different views.
7. To customize a view:
    a. First the report definition must be saved outside of the Dionex Template folder. This is done by right clicking on the page and selecting “Save Report Definition”. SAVE THE FILE OUTSIDE OF THE LOCKED DIONEX TEMPLATE FOLDER.
    b. The chromatogram can now be customized as desired.
    c. To change the columns displaying integration values, you must enter Layout Mode.

i. From the "Edit" Menu, select "Layout Mode".

ii. In this mode, you can change the parameters of the report columns, by double clicking on the report column name. This displays all available parameters.



8. Once the report definition is customized as desired, right click and again save the report definition.
9. For advanced customization options, please consider attending a Dionex training course.

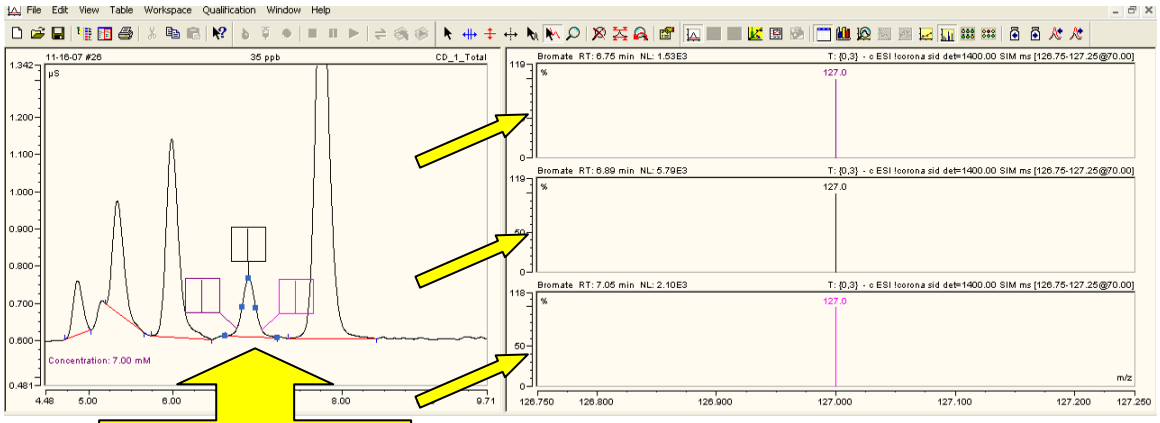
7.1 How to make a report definition for several SIM channels on one page

1. Open a finished sample run.
2. At the top of the screen go to the "View" Menu. Select "Printer Layout".
3. On the printer layout screen, go to the "Workspace" Menu. Select "Load Report Definition...".
4. In the browsing window that opens, scroll up to the Dionex Templates folder. Open this folder and then select the "Reports" folder. Inside the reports folder, select "Peak Summary Report".
5. The screen will open back up to the integration view. Go back to the "View" menu and select "Printer Layout".
6. Go up to the "Workspace" menu. Select "Save Report Definition…" and save this report somewhere outside of the locked Dionex templates folder.
7. Go up to the Edit menu and select "Layout Mode". Once the report is in layout mode, you will see little tabs in the corners of the headers.
8. Right click on the column for "Retention Time". Select "Report Column Properties" from the menu list.
9. In the menu box that appears, focus on the boxes in the lower portion of the screen. Since all of the SIM channels are linked to a specific analyte, change:
    a. The "Peak" box from the default of "Selected Peak" → "Fixed Peak" and choose the name from the pull down arrow. (The name comes from the associated .qnt file.)
    b. The "Channel" box from the default "Selected Channel" to "Fixed Channel". Choose the SIM channel that associates with the name of the peak for "Fixed Peak" (a).
10. Repeat the above steps for all other parameters of the table (height, area, amount, concentration).

11. Go up to the “Workspace” menu. Select “Save Report Definition…”. You can overwrite the existing file. (you may want to save after each step you are happy with, since there is no “undo” function.)
12. Copy the table that was just created, and paste an identical one right below it.
13. Modify each column as above for the so the specific SIM channel and Peak Name are aligned.
14. Minimize the rows with the additional headers so it looks like it is in one big table.

7.2 Removing the Retention Spectra

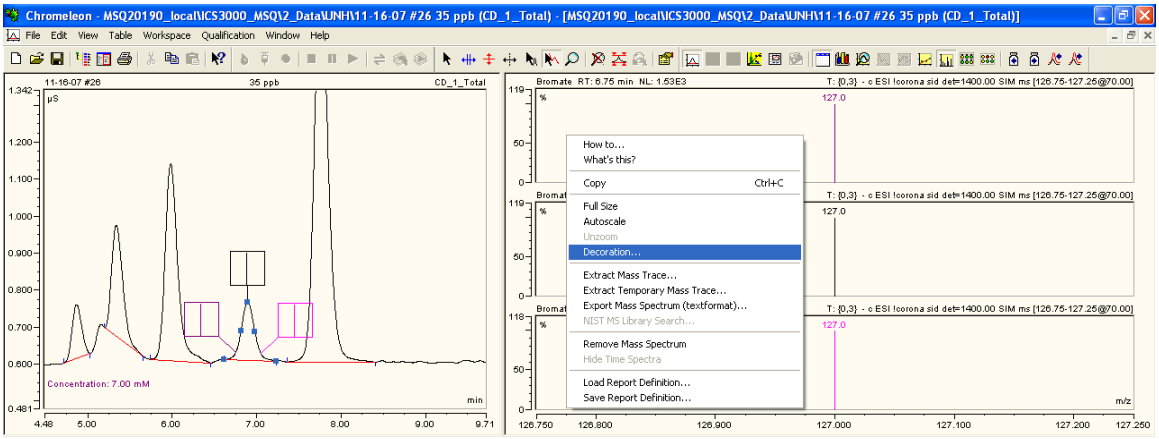
Retention spectra appear the first time you click on the peak to find the mass spectra. They are seen as a split screen on the right side of the page. Their purpose is to show the spectra of the chromatography peak at the beginning, middle, and end of the peak. At times, these may act as more of a hindrance than a help.


Retention spectra are seen as three boxes around the peak in the CD/UV trace and three separate traces on the spectra side of the screen.

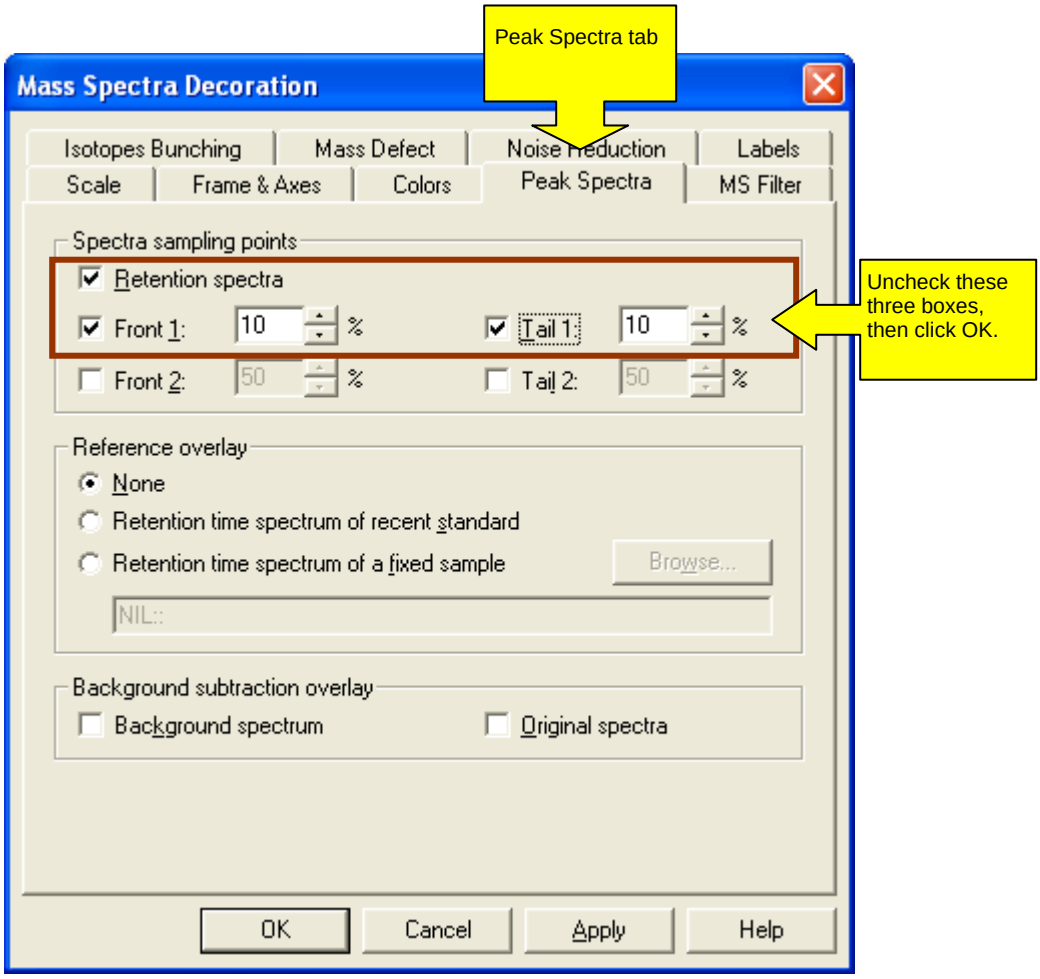
To remove the retention spectra:

1. Right click on the spectra side of the screen.
2. Select “Decoration…”.
3. Go to the Peak Spectra tab.
4. Uncheck the boxes next to “Retention spectra”, “Front 1”, and “Tail 1”.
5. Click OK.

6. At this point, if the spectrum does not appear in the spectra screen, select the portion of the chromatogram where you would like to look at the spectra.



7. To save this layout, go to Workspace and save the report definition.



Establishing NIST 2.0 Anion Library

8.1 Introduction

This section describes the installation procedure for the **Chromeleon Anion MS Spectral Library (Anion MS Library)**, and how to use the Anion MS Library to perform spectra search for unknown analytes. The Anion MS Library currently contains single quadrupole MS spectra of eighty-four common anions and organic acids.

It is not intended to explore the complete functionalities and features of the NIST 2.0 MS Search. For detailed information on how to use the NIST 2.0 MS Search and additional information, please refer to the NIST User's Manual which can be downloaded from this link:

http://www.nist.gov/data/WebGuide/nist01/Ver20Man.pdf

8.2 Parameters Used to Acquire Standard Spectra

Ion Chromatography

System :	ICS-2000 Reagent-Free Ion Chromatography (RFIC)
Column:	System AS20 (P/N 063065) or AS11-HC (P/N 052961) hydroxide selective column (2 × 250 mm)
Suppressor:	ASRS 300 (P/N 064555), external water mode
Assistant make up flow:	0.2 mL/min acetonitrile delivered by an AXP-MS pump (P/N 060684). Static mixing tee (P/N 063143) for combining IC and organic make up flow
Detection:	Suppressed conductivity and MSQ Plus mass spectrometer

Mass Spectrometry

System:	MSQ Plus single quadrupole mass spectrometer
Ionization interface:	Electrospray ionization (ESI)
Probe temperature:	450 °C

Needle voltage:	1500 V
Cone Voltage:	50 V
Nebulizer gas:	Nitrogen at 85 psi

All MS spectra were obtained from chromatographically separated peaks to eliminate interference from impurities, at the specified MS conditions. THE RELATIVE INTENSITIES OF MS PEAKS STRONGLY DEPEND ON THE CONE VOLTAGE SETTINGS and also may vary slightly due to the different operating environments. Thus for the best result, it is highly recommended to acquire MS spectra at the above specified conditions (especially the cone voltage) for the best search accuracy, or establish a customized user library using your daily operating conditions.

NOTE: The Dionex Anion Library only contains MS Spectra using an ESI interface which is the primary interface for IC-MS applications. Users may build their own APCI library following the steps described in Section 9.3.

Instrument control, data acquisition, and processing were achieved through Chromeleon chromatography data system software (version 6.8, SR6). MSQ Plus was calibrated and tuned through Xcalibur (version 2.0.7) and MSQ 2.0 SP1 tune program.

8.3 System Requirements

PCs preinstalled with Chromeleon and Xcalibur are ready for NIST MS Search program installation.

NOTE: Chromeleon 6.8 SR6 and Xcalibur 2.0.7 with MSQ 2.0 SP1 programs are fully tested with the NIST MS Search and thus are recommended versions.

Chromeleon 7.0 is not currently compatible with MS functionality and can not be used for MS instrument control and MS spectra search.

8.4 Installation of NIST MS Search and Updates

NIST MS Search program (current version 2.0) is required to be preinstalled to enable the MS library search in Chromeleon. The NIST MS Search program upgrade is also required to enable building or installing a new/customized user library.

> **NOTE:** The NIST MS Search program may already be installed on your system. To check for the program, from the Windows desktop select "My Computer" and select the "Add or Remove Programs" option. A list will be populated with the programs installed on your PC. If the NIST MS Search is loaded, it will be listed as "NIST MS SEARCH and AMDIS".

8.5 Introduction to NIST Search Program and Libraries

The NIST MS Search program is a freeware application package developed by the National Institute of Standards and Technology (NIST). It can take MS spectra acquired by different instruments, compare with the NIST/EPA/NIH standard spectra database or user customized database for identification and confirmation purposes.

The search program comes with a preloaded GC-EI-MS spectra database upon installation. The reason EI spectra were chosen for these libraries is that it was the method of choice for GC and there was only the one main parameter – the EI setting. Changing EI can skew the spectrum enormously, so it was forced to one value for all – 70eV. For liquid chromatographic applications, Atmospheric Pressure Ionization (API) interfaces are often used to couple chromatography and mass spectrometry systems. ESI is a very soft ionization technique and usually produces a single main ion. If you want to create fragments from an API spectrum using collision induced dissociation (CID) with the Cone Voltage, there is the problem that fragmentation is method and instrument dependent and in most cases substantially different from the spectra produced by EI. Due to the different ionization mechanisms of interfaces coupling GC and LC to MS, the GC-EI-MS library is not applicable for LC-MS spectra search. Users wishing to library search API spectra should make their own libraries.

8.6 Install NIST MS Search Program

The NIST MS Search program can be installed from the Xcalibur software CD shipped with the MSQ Plus instrument. Contact your Dionex service representative to obtain the recommended versions of software if you don't have them available. Possible additional charges may apply for Chromeleon and Xcalibur upgrades.

To install the MS Search program:

1. Insert the Xcalibur software CD to start the installation.
2. From the pop-up window (shown in Figure 1), select the "NIST 2.0" and follow the on-screen instructions to finish installing the NIST MS Search.

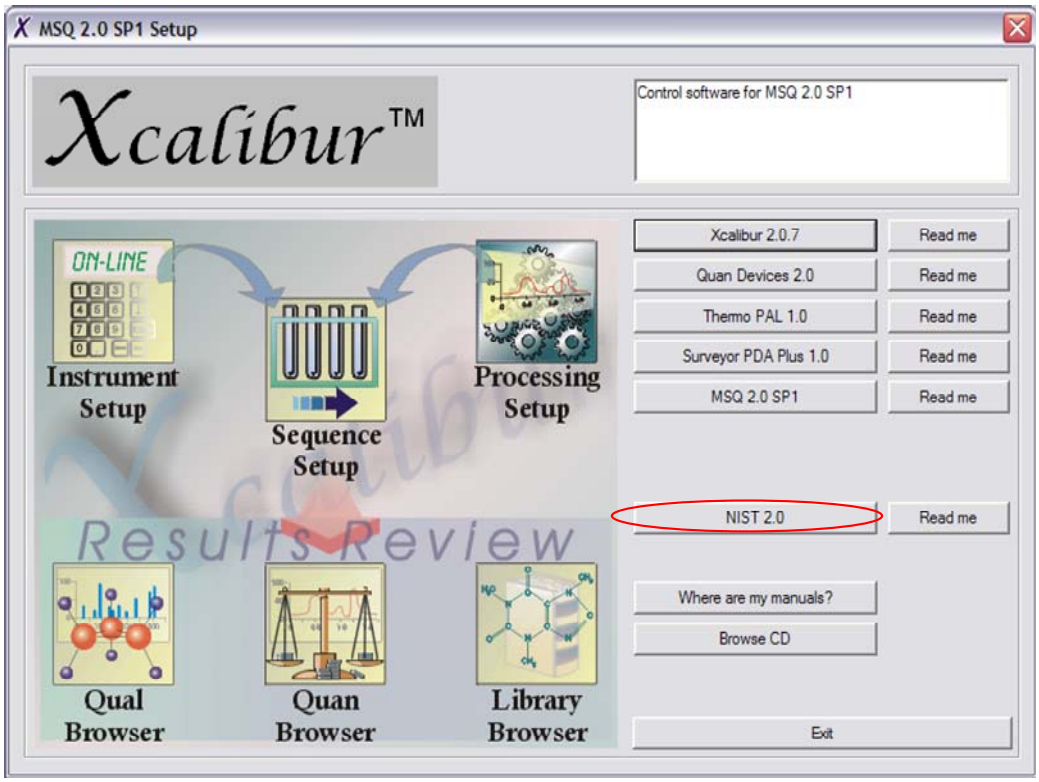


Figure 1. Install NIST 2.0 from Xcalibur 2.0 Installation CD.

The default installation folder for the NIST MS Search program is:

C:\Program Files\NISTMS\MSSEARCH.

8.7 Download and Install NIST MS Search Program Upgrade

1. Download the upgrade from this link (as shown in Figure 2):

   http://chemdata.nist.gov/mass-spc/Srch_v1.7/index.html#NIST_02_MS_Library_UPDATE,

.

2. Save the upgrade program on your local PC hard drive, e.g. *desktop*, the default file name is **V17_20_UPG**.

MS Search Program v.2.0 UPGRADE

This upgrade installs version 2.0 of the NIST MS Search Program. This upgrade will not replace your NIST MS Search Program v 1.6 or 1.7, but it will upgrade any previously distributed version 2.0 files. Please refer to readme_v20.txt included in the zip file below for more details. ***This software upgrade should not be applied to the software accompanying the* NIST 02 MS Library.**

UPGRADE INSTRUCTIONS: Detailed instructions are included in readme_v20.txt in the zip file. Please read this document before downloading the complete zip file. For this upgrade to work properly, it is important that the MS Search Program is not open when the files are extracted.

Download MS Search Program v.2.0 Upgrade

Figure 2. Download MS Search program upgrade.

3. Close the NIST MS Search program if it is already running.
4. Extract upgrade files from the upgrade zip package "V17_20_UPG" to the default folder of the MS Search program, i.e. "*C:\Program Files\NISTMS\MSSEARCH*".
5. To ensure the upgrade is properly installed. Run the MS Search program from the Microsoft Start Menu → Programs → NIST MS Search and AMDIS → MS Search v.2.0.

NOTE: For convenience, you can also create a desktop shortcut of the program by right-clicking the MS Search v.2.0 from start menu and select "Send to → Desktop (create shortcut)".

6. After launching the MS Search program, select the "Librarian" tab on the bottom of the screen, shown in Figure 3. If the "Create Library" button shows up in the toolbar, the upgrade has been successfully installed.

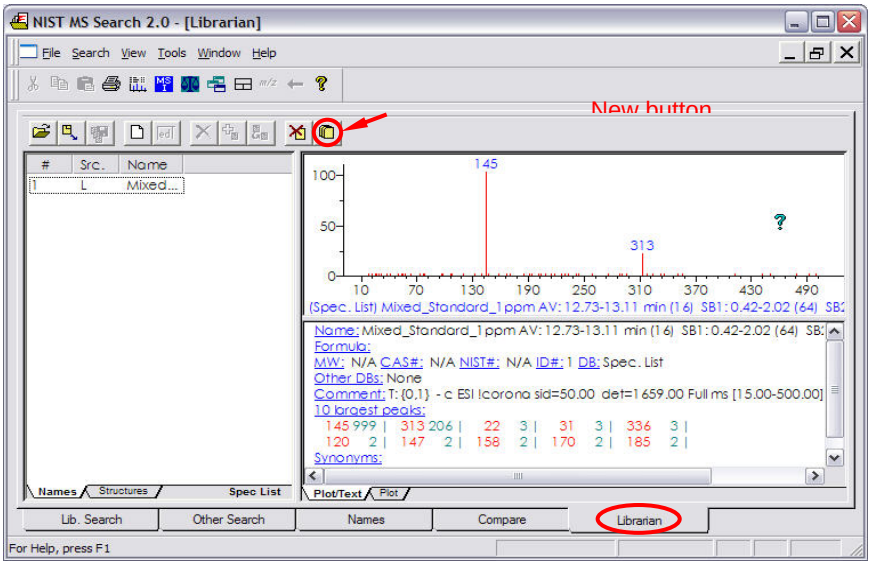

NIST MS Search 2.0 - [Librarian]
File Search View Tools Window Help
New button
Src. Name
1 L Mixed...
145
313
100
50
0
10 70 130 190 250 310 370 430 490
(Spec. List) Mixed_Standard_1 ppm AV: 12.73-13.11 min (16) SB1: 0.42-2.02 (64) SB:
Name: Mixed_Standard_1 ppm AV: 12.73-13.11 min (16) SB1: 0.42-2.02 (64) SB:
Formula:
MW: N/A CAS#: N/A NIST#: N/A ID#: 1 DB: Spec. List
Other DBs: None
Comment: T: (0,1) - c ESI Icorona sid=50.00 det=1659.00 Full ms [150.00-500.00]
10 largest peaks:
145 999 | 313 206 | 22 3 | 31 3 | 336 3 |
120 2 | 147 2 | 158 2 | 170 2 | 185 2 |
Synonyms:
Names Structures Spec List
Plot/Text Plot
Lib. Search Other Search Names Compare Librarian
For Help, press F1

Figure 3. Ensure the successful installation of the NIST MS Search Upgrade.

8.8 Importing the IC-MS Anion Library

MS Spectra and structure information for selected anions are packaged in the "Dionex IC-MS Anion" zip file. This package is available from your local Dionex representative and will be available from the Dionex website (www.dionex.com) in the near future.

1. Click the circled button "Create Library" in Figure 3 to create a new library with the name "IC-MS Anions".
2. Create a temporary folder on the PC desktop with name "Temporary Library Files" and extract the files (a total of 23 files) from "Dionex IC-MS Anions" to this temporary folder.
3. Locate the library folder named "IC-MS Anions" in "*C:\Program Files\NISTMS\MSSEARCH*".
4. Close the NIST MS Search program.
5. Copy all 23 files in the "Temporary Library Files" folder to the NIST library folder created by the MS Search program which will be located in "C:\Program Files\NISTMS\MSSEARCH\IC-MS Anions". When a "Confirm file replace" window pops up, click "Yes to All" to replace all old files with the new library files containing anion spectra.

8.9 Specify Library Search Options

1. Start the MS Search program again from the desktop shortcut or from the Windows Start menu.
2. Select "Library Search Options" from the "Tool" drop down menu from the top tool bar.
3. As shown in Figure 4, click on the 2nd tab of the dialog window named "Libraries". From the left side of the window, click "ic-ms anions" from "available libs" and click ">>Add>>" to "Included libs".
4. Click on the "nistdemo" from the "Included libs" and click on the delete button located next to "Included libs" to delete the NIST Demo library which contains GC-EI-MS spectra not applicable to IC-MS applications. Click "OK" to save the settings.

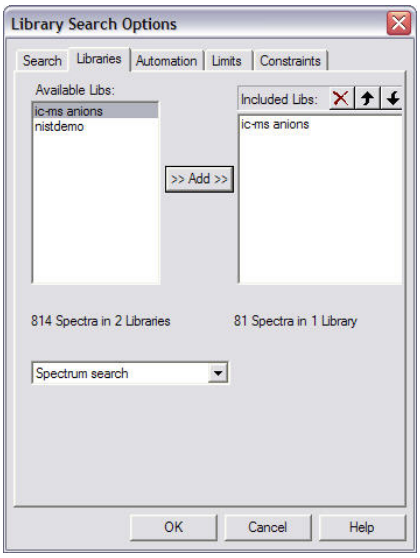


Figure 4. Specify the Search Library

NOTE: If multiple applicable libraries are available, for example, a Dionex "IC-MS Anions" and a user customized library, both libraries can be added to the "Included Libs:". The libraries selected will be searched sequentially, and if a representative compound is in one or more libraries, it may appear multiple times in the results of a search. This can be used to an advantage, for instance, if a compound was run twice with different parameters, the search will return the closest matches, ranking the selected spectra with the best fit to the searched spectrum.

Using the NIST MS Search with Chromeleon

9.1 Search Chromeleon MS spectrum against NIST Libraries

1. To search an acquired MS spectrum, double click the sample from the sequence to open the data.
2. Click on “Show MS Spectra” button from the toolbar, as shown in Figure 5, to view the MS spectra on screen. Select “Automatic Tool” to click the desired peak or use “Spectra/Plots Tool” to select the time range to display the MS spectra.

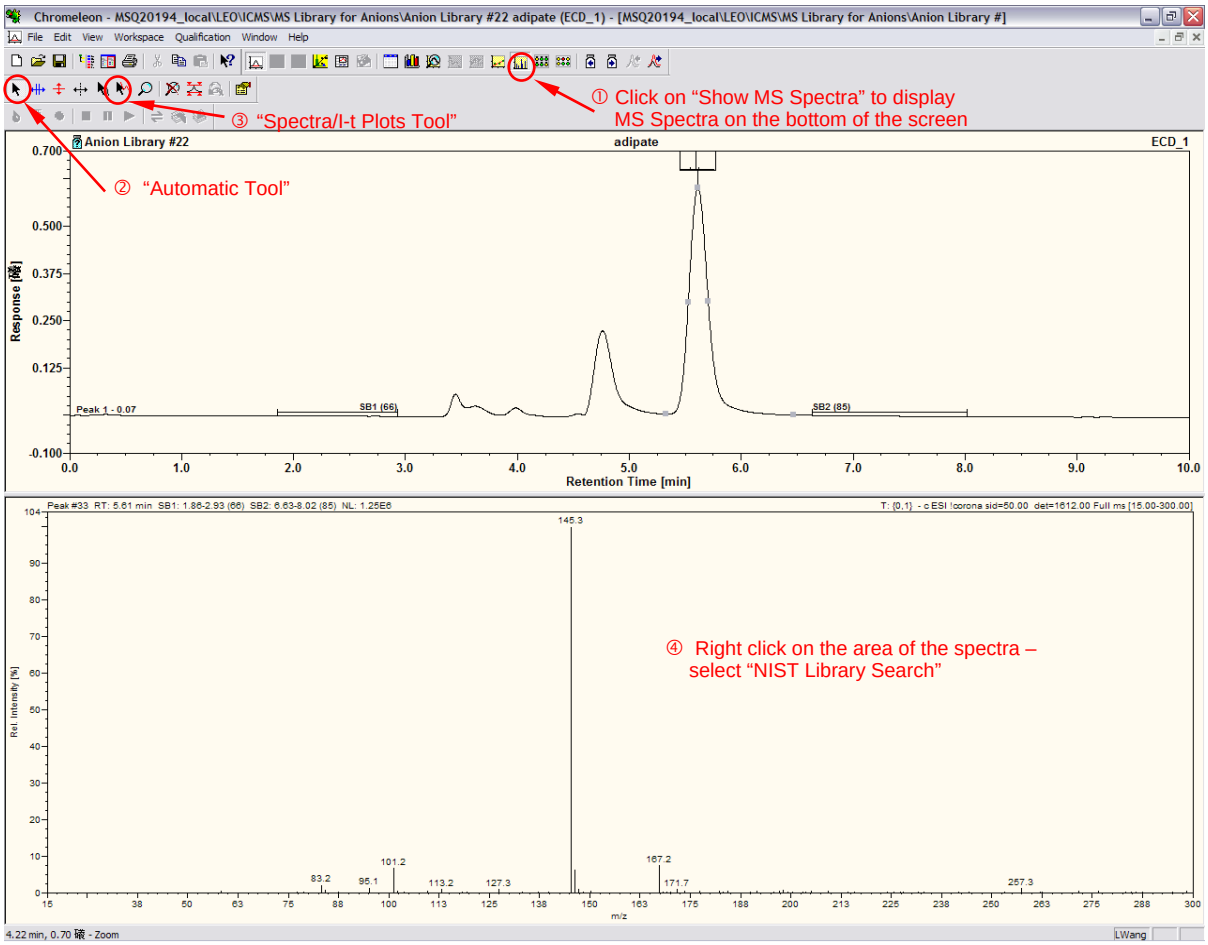

① Click on “Show MS Spectra” to display MS Spectra on the bottom of the screen
② “Automatic Tool”
③ “Spectra/I-t Plots Tool”
④ Right click on the area of the spectra – select “NIST Library Search”
Anion Library #22
adipate
ECD_1
Response [pA]
Retention Time [min]
Peak 1 - 0.07
SB1 (66)
SB2 (85)

Figure 5. Viewing an MS Spectrum in Chromeleon.

3. Right click on the MS spectra and select “NIST MS Library Search”; this action will automatically launch the NIST MS Search program if it is not running.
4. Leave the “Enable” “Mass Defect” unchecked, and click “OK” to continue.
5. The search result will be shown in the NIST MS Search program, as shown in Figure 6 (the default view, here, an adipate standard is used for the demonstration). The hits are sorted by probability on the bottom right corner. Additional information is also available such as the spectra of the unknown and the top hit analyte in the library.

NOTE: For additional information about the features of displaying and manipulating the form and format of the NIST MS Search display, refer to the “Help” section of the program.

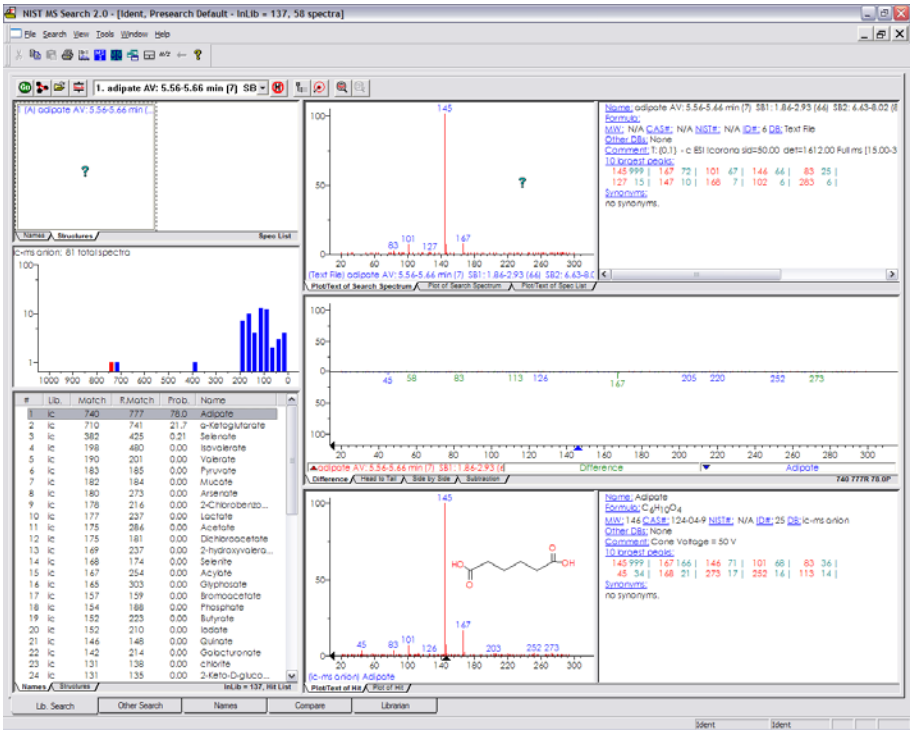

NIST MS Search 2.0 - [Ident, Presearch Default - InLib = 137, 58 spectra]
File Search View Tools Window Help
1. adipate AV: 5.56-5.66 min (7) SB
Lib. Search
Other Search
Names
Compare
Librarian

Figure 6. Search Result in NIST MS Search Program.

9.2 Adding a Chromeleon MS spectra to an Existing Library

A spectrum can be added to an existing library, e.g. the Dionex Anion Library, as a new entry or a replacement of an existing entry.

1. Perform the library search as described in "Search MS spectra from Chromeleon against NIST Library" section.
2. Click on the "Librarian" tab on the bottom tab bar and navigate to the library entry window.
3. Select the analyte by clicking on the analyte listed in the left panel, and click on the "Edit Spectra" button from the toolbar, as shown in Figure 7.
4. Enter all known related information of the analyte spectrum, such as name, formula, molecular weight, and CAS number. To replace an existing entry, type in the same "Name" as the entry you want to replace.
5. Select "Add to Library" after entering related spectrum information and select the desired library when a pop up window shows up asking which library to add into, and click "OK" to finish spectrum entry.

NOTE: To add a structure to a library entry, special software is required to draw the chemical structure, e.g. ChemDraw; or use an existing chemical structure file with the file extension: .MOL or .SDF. The structure display is an option and is not required to make an entry in the library.

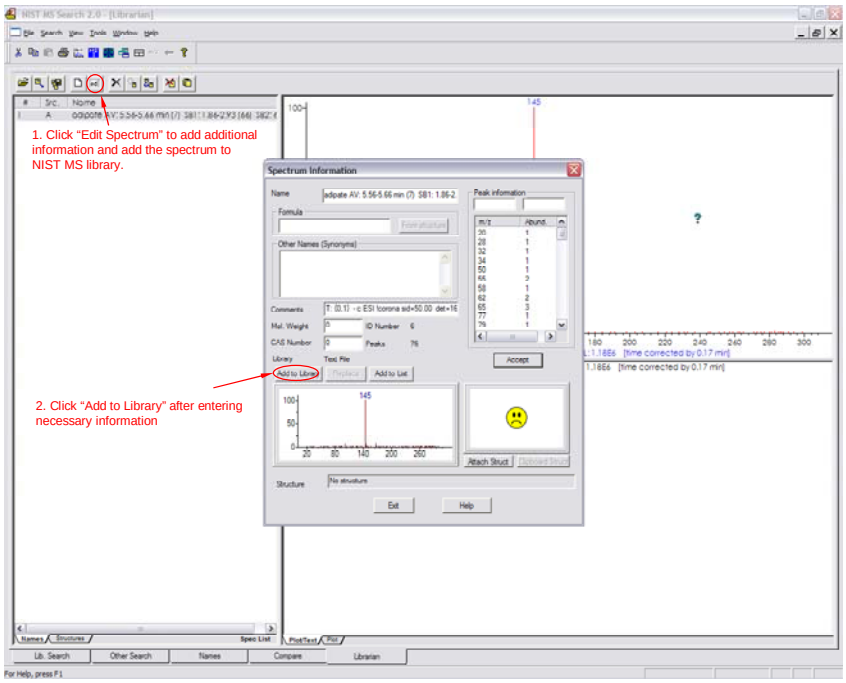

1. Click "Edit Spectrum" to add additional information and add the spectrum to NIST MS library.
2. Click "Add to Library" after entering necessary information
Spectrum Information

Figure 7. Add MS Spectra to NIST MS Library.

9.3 Create Your Own Library and Add Entries

Users can create their own libraries in the NIST MS Search program. This function is useful when the analyte of interest is not included in the existing library or spectra at specific conditions are not available in the existing library, e.g. spectra at high cone voltage for structural information.

To add a new library, click the circled button “Create Library” in Figure 3 to create a new library and specify the name of library. Follow section 3.2 to add new spectra to this library.

For more information of the NIST functions described in this manual and additional functions, refer to the NIST MS Search Program user’s manual.